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(54) Title: CHIMERIC INFECTIOUS BURSAL DISEASE VIRUS cDNA CLONES, EXPRESSION PRODUCTS AND VACCINES BASED THEREON (57) Abstract Chimeric cDNA for the expression of immunogenic polypeptides include the genetic epitopic determinants for a base infectious bursal disease virus strain and at least one other infectious bursal disease virus strain. The genetic epitopic determinants encode amino acids or amino acid sequences which define epitopes bound to by previously established monoclonal antibodies. The immunogens expressed by the cDNA may be employed to provide a vaccine against a plurality of IBDV strains. The epitopic determinant of IBDV lethal strains has been detected, and an immunogen for conferring immunity with respect thereto is disclosed. Similarly, a monoclonal antibody specific for IBDV lethal strains is identified, and a vaccine for passive immunization therewith is also disclosed. Immunogens exhibiting conformational epitopes, in the form of virus-like particles, are effective in the preparation of vaccines.		

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CHIMERIC INFECTIOUS BURSAL DISEASE VIRUS cDNA CLONES,
EXPRESSION PRODUCTS AND VACCINES BASED THEREON

Abstract:

Abstract of WO9526196

Chimeric cDNA for the expression of immunogenic polypeptides include the genetic epitopic determinants for a base infectious bursal disease virus strain and at least one other infectious bursal disease virus strain. The genetic epitopic determinants encode amino acids or amino acid sequences which define epitopes bound to by previously established monoclonal antibodies. The immunogens expressed by the cDNA may be employed to provide a vaccine against a plurality of IBDV strains. The epitopic determinant of IBDV lethal strains has been detected, and an immunogen for conferring immunity with respect thereto is disclosed. Similarly, a monoclonal antibody specific for IBDV lethal strains is identified, and a vaccine for passive immunization therewith is also disclosed. Immunogens exhibiting conformational epitopes, in the form of virus-like particles, are effective in the preparation of vaccines. Data supplied from the esp@cenet database - Worldwide

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Description

Chimeric Infectious Bursal Disease Virus cDNA Clones,
Expression Products and Vaccines Based Thereon

Technical Field:

The present invention provides chimeric IBDV immunogens which actively protect against virulent and lethal challenge by Classic and variant IBDV strains, and methods for obtaining vaccines containing these chimeric immunogens and vaccines.

Background Art

Infectious bursal disease virus (IBDV) is responsible for a highly contagious immunosuppressive disease in young chickens which causes significant losses to the poultry industry worldwide (reviewed in Kibenge (1988) "J. Gen. Virol.", 69:1757-1775). Infection of susceptible chickens with virulent IBDV strains can lead to a highly contagious immunosuppressive condition known as infectious bursal disease (IBD). Damage caused to the lymphoid follicles of the bursa of Fabricius and spleen can exacerbate infections caused by other agents and reduce a chicken's ability to respond to vaccination as well (Cosgrove (1962) "Avian Dis.", 6:385-3894).

There are two serotypes of IBDV (McFerran et al (1980) "Avian Path." 9:395-404). Serotype 1 viruses are pathogenic to chickens and differ markedly in their virulence (Winterfield et al (1978) "Avian Dis." 5:253-260), whereas serotype 2 viruses, isolated from turkeys, are avirulent for chickens (Ismail et al (1988) "Avian Dis.", 32:757-759; Kibenge (1991) "Virology" 184:437-440).

IBDV is a member of the Birnaviridae family and its genome consists of two segments of double-stranded RNA (Dobos et al (1979) "J. Virol.", 32:593-605). The smaller segment B (~2800bp) encodes VP1, the dsRNA polymerase. The larger genomic segment A (~3000bp) encodes a 110 kDa precursor polyprotein in a single open reading frame (ORF) that is processed into mature VP2, VP3 and VP4 (Azad et al (1985)

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"Virology" 143:35-44). From a small ORF partly overlapping with the polyprotein ORF, segment A can also encode VP5, a 17 Kda protein of unknown function (Kibenge et al (1991) "J. Gen. Virol.", 71:569-577).

While VP2 and VP3 are the major structural proteins of the virion, VP2 is the major host-protective immunogen and causes induction of neutralizing antibodies (Becht et al (1988) "J. Gen. Virol." 69:631-640; Fahey et al (1989) "J. Gen. Virol.", 70:1473-1481). VP3 is considered to be a group-specific antigen because it is recognized by monoclonal antibodies (Mabs) directed against VP3 from strains of both serotype 1 and 2 (Becht et al (1988) "J. Gen. Virol.", 69:631-640). VP4 is a virus-coded protease and is involved in the processing of the precursor protein (Jagadish et al (1988) "J. Virol.", 62: 1084-1087).

In the past, control of IBDV infection in young chickens has been achieved by live vaccination with avirulent strains, or principally by the transfer of maternal antibody induced by the administration of live and killed IBDV vaccines to breeder hens. Unfortunately, in recent years, virulent variant strains of IBDV have been isolated from vaccinated flocks in the United States (Snyder et al (1988b) "Avian Dis.", 32:535-539; Van der Marel et al (1990) "Dtsch. Tierarztl. Wschr.", 97:81-83). The use of a select panel of Mabs, raised against various strains of IBDV, has led to the identification of naturally occurring GLS, DS326, RS593 and Delaware variant viruses in the United States. Substantial economic losses have been sustained due to the emergence of these antigenic variants (Delaware and GLS) in the field (Snyder et al (1992) "Arch. Virol.", 127:89-101), copending U.S. Application Serial No. 08/216,841, filed March 24, 1994, Attorney Docket No. 2747-053-27, Snyder, copending herewith). These variant strains are antigenically different from the Classic strains of IBDV most typically isolated before 1985, and lack epitope(s) defined by neutralizing monoclonal antibodies

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(Mabs) B69 and R63 (Snyder et al (1988a) "Avian Dis.", 32:527-534; Snyder et al (1998b) "Avian Dis.", 32:535-539; Snyder et al (1992) "Arch. Virol.", 127:89-101). Since the appearance of these variant strains in the field, many commercially available live and killed vaccines for IBDV have been reformulated in an attempt to better match the greater antigenic spectrum of viruses recognized to be circulating in the field.

Efforts to develop a recombinant vaccine for IBDV have been made, and the genome of IBDV has been cloned (Azad et al (1985) "Virology", 143:35-44). The VP2 gene of IBDV has been cloned and expressed in yeast (Macreadie et al (1990) "Vaccine", 8:549-552), as well as in a recombinant fowlpox virus (Bayliss et al (1991) "Arch. Virol.", 120:193-205). When chickens were immunized with the VP2 antigen expressed from yeast, antisera afforded passive protection in chickens against IBDV infection. When used in active immunization studies, the fowlpox virus-vectored VP2 antigen afforded protection against mortality, but not against damage to the bursa of *Fabricius*.

Recently, the synthesis of VP2, VP3 and VP4 structural proteins of the variant GLS IBDV strain in a *baculovirus* expression system has been described (Vakharia et al (1993) "J. Gen. Virol.", 74:1201-1206). In an initial two dose active immunity study in SPF chickens, *baculovirus* expressed GLS proteins were able to confer 79% protection against virulent GLS challenge (Vakharia et al (1993) "J. Gen. Virol.", 74:1201-1206). In a subsequent extended study of active cross-immunity, by increasing the antigenic mass of the *baculovirus* expressed GLS protein, complete protection against the variant GLS and E/Del strains was obtained with a single dose, but only partial protection was afforded against the Classic STC strain unless two doses were administered.

In recent years, the complete, nucleotide sequences of the large segment A of five serotype 1 IBDV strains; 002-73

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(Hudson et al (1986) "Nucleic Acids Res." 14:001-5012), Cu-1, PBG98, 52/70 (Bayliss et al (1990) "J. Gen. Virol.", 71:1303-1312), STC (Kibenge (1990) "J. Gen. Virol.", 71:569-577), and serotype 2 OH strain (Kibenge (1991) "Virology", 184:437-440) have been determined. In addition, the VP2 gene of virulent Japanese IBDV strains (Lin et al (1993) "Avian Dis.", 37:315-323) and Delaware variants A and E (Lana et al (1992) "Virus Genes" 6:247-259; Heine et al (1991) "J. Gen. Virol.", 72:1835-1843) has been sequenced. However, no one has completely cloned and characterized the entire long segment of any United States IBDV variant.

Disclosure of the Invention

Inventors have now identified the region of the IBDV genome which is responsible for antigenic variation. A DNA sequence containing the central variable region of VP2 protein, as well as a plasmid incorporating the same, have been constructed. This DNA sequence can be manipulated to generate desired virus neutralizing epitopes or immunogenic polypeptides of any IBDV strain. In turn, these immunogenic segments can be incorporated into new recombinant IBDV vaccines.

Brief Description of the Drawings

Figure 1 illustrates the construction of various chimeric plasmids encoding IBDV-specific polyproteins. A map of the IBDV genome with its coding regions is shown at the top of the Figure. Selected restriction sites are incorporated in the Figure: B, BamHI; E, BstEII; N, NdeI; R, NarI; S, SpeI. Dashes indicated the substitution of the D78 sequence (NdeI-NarI fragment) into the GLS sequence to restore the B69 epitope region. Solid line and dotted line indicate the substitution of the E/Del-22 and DS326 sequences, respectively, into the GLS sequence to restore the B63 epitope region or to delete the 179 epitope region, respectively.

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Figure 2 is electron micrographs of IBDV virus-like particles (|—|) = 100nm). A. Actual empty particles (without RNA) from purified virus. B. Virus-like particles (empty capsids) derived from a recombinant *baculovirus* expressing the large genome segment of IBDV in insect cells

Figure 3 is a comparison of the deduced amino acid sequences of the structural proteins (VP2, VP3 and VP4) of ten IBDV strains. Dashes (-) indicate amino acid identity and crosses (x) denote a region where the sequence was not determined. Filled bar (■) indicates a gap in the sequence and vertical arrowheads (↓) mark the possible cleavage sites of VP2/VP4 and VP4/VP3. The two hydrophilic peaks in the variable region are overlined.

Figure 4 is a phylogenetic tree for the IBDV structural proteins using the PAUP (phylogenetic analysis using parsimony) version 3.0 program (Illinois Natural History Survey, Champaign, Illinois).

Figure 5 reflects the DNA and amino acid sequence for the GLS virus structural protein fragment VP2/VP4/VP3. A vertical line indicates the start/stop points for the VP2, VP4 and VP3 regions.

Figure 6 reflects the DNA and amino acid sequence for the E/Del 22 virus structural protein fragment VP2/VP4/VP3.

Figure 7 is a table of the amino acid identities for key locations (epitopic determinants) of eight different IBDV.

Definitions:

IBD - infectious bursal disease as described above.

IBDV - infectious bursal disease virus, a virus capable of, at a minimum, inducing lesions in the bursa of *Fabricius* in infected poultry.

Epitopic Determinants - amino acids or amino acid sequences which correspond to epitopes recognized by one or more monoclonal antibodies. Presence of the amino acid or amino acid sequence at the proper ORF location causes the

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polypeptide to exhibit the corresponding epitope. An epitopic determinant is identified by amino acid(s) identity and sequence location.

Genetic Epitopic Determinants - nucleotide sequences of the ORF which encode epitopic determinants.

Conformational Epitopes - epitopes induced, in part or in whole, by the quaternary (three-dimensional) structure of an IBDV polypeptide. Conformational epitopes may strengthen binding between an IBDV and a monoclonal antibody, or induce binding whereas the same sequence, lacking the conformational epitope, would not induce binding between the antibody and the IBDV polypeptide at all.

Virus-Like Particles - three-dimensional particles of natural or recombinant amino acid sequences mimicking the three-dimensional structure of IBDV (encoded by the large genome segment A) but lacking viral RNA. Virus-like particles exhibit conformational epitopes exhibited by native viruses of similar sequence. Virus-like particles are created by the proper expression of DNA encoding VP2, VP4, VP3 structural proteins in a proper ORF.

Epitopic Determinant Region - Limited region of amino acid sequence of VP2 of IBDV that is replete with epitopic determinants, variation among amino acids of this limited region giving rise to a high number of epitopes recognized by different monoclonal antibodies.

Best Mode for Carrying Out the Invention

Recombinant, immunogenic polypeptides exhibiting the epitopes of two or more native IBDV, as well as recombinant virus-like particles exhibiting the epitopes of two or more native IBDV and conformational epitopes are effective immunogens for vaccines which can be used to confer protection against a wide variety of IBDV challenge in inoculated poultry. The recombinant polypeptides and virus-like particles are obtained by the expression of chimeric DNA

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prepared by the insertion, in the VP2 region of a base IBDV, of epitopic determinants for at least a second IBDV. This is most easily done by substitution of the genetic epitopic determinants for the amino acids identities and locations reflected in Figure 7. Thus, where the epitopic determinant of the second IBDV differs from that of the base IBDV, the genetic epitopic determinant for the differing second IBDV is inserted in place of the genetic epitopic determinant at that location of the base IBDV. An example, combining epitopic determinants from the D78, E/Del 22 and DS326 IBDV into the base GLS IBDV is set forth in Figure 1. Thus, one DNA sequence can be prepared with genetic epitopic determinants for a plurality of native IBDV. These recombinant plasmids can be inserted into a variety of packaging/expression vector, including *baculovirus*, fowlpox virus, Herpes virus of turkeys, adenovirus and similar transfection vectors. The vectors can be used to infect conventional expression cells, such as SF9 cells, chicken embryo fibroblast cell lines, chicken embryo kidney cells, vero cells and similar expression vehicles. Methods of transfection, and methods of expression, as well as plasmid insertion into transfection vehicles, are well known and do not constitute an aspect of the invention, per se.

The expression of the chimeric cDNA of the invention generate immunogenic polypeptides which reflect epitopes of a plurality of native IBDV, and the expression of a recombinant VP2, VP4, VP3 cDNA segment, with the VP2 region again comprising genetic epitopic determinants for at least two native IBDV give rise to immunogenic virus-like particles.

The immunogenic polypeptides and virus-like particles can be harvested using conventional techniques (Dobos et al, "J. Virol.", 32:593-605 (1979)). The polypeptides and virus-like particles can be used to prepare vaccines which will confer protection on inoculated poultry, in particular, chickens, and in a preferred embodiment, broiler chickens, protection against challenge from each IBDV bearing an epitope reflected

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in the plurality of epitopic determinants present in the inoculum. Thus, a single immunogen gives rise to immunity against a variety of IBDV, each IBDV whose genetic epitopic determinant has been incorporated in the chimeric cDNA.

The administration of the vaccines can be effectively done according to well-established procedures. In U.S. Patent 5,064,646, which is incorporated herein by reference, methods are described for the effective inoculation of chicks based on the then novel isolation of GLS IBDV. Similar administration and dosage regimens can be employed herein. Since the polypeptides and virus-like particles lack viral RNA, they are avirulent. The vaccines may therefor be prepared by simple incorporation of the immunogenic polypeptides and virus-like particles in a pharmaceutical carrier, typically a suspension or mixture. Appropriate dosage values are best determined through routine trial and error techniques, given the different antibody titers induced and/or the quantity of different epitopes present which will induce complete cross-immunity to virulent challenge. In general, pharmacologically acceptable carriers such as a phosphate buffered saline, cell culture medium, Marek's virus vaccine diluent oil adjuvants and other adjuvants, etc., can be used. Administration is preferably done to hens entering egg laying periods which provides induction of antibody which is passively transferred through the egg to the chick to prevent early infection by virulent field strength IBDV. Conversely, the recombinant vaccine may be delivered in a replicating vector at any time in a chicken's life span, preferably at one day of age. Experience has demonstrated that, generally, that the level of protection may be improved by a second inoculation.

This invention may be further understood by reference to the specific examples set forth below.

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Examples:**Background Methodology**

To determine the molecular basis of antigenic variation in IBDV, the genomic segment A of four IBDV strains: GLS, DS326, Delaware variant E (E/Del) and D78 was cloned and characterized by sequencing. By comparing the deduced amino acid sequences of these strains with other serotype 1 and 2 sequences published previously, the putative amino acid residues involved in the binding with various neutralizing Mabs were identified, and the phylogenetic relationship of IBDV structural proteins was examined.

GLS, DS326 and STC strains of IBDV were propagated in the bursa of specific-pathogen-free chickens (SPAFAS, Inc., Norwich, CT, USA). Tissue culture adapted E/Del-22, D78 and OH (serotype 2) strains of IBDV were propagated in primary chicken embryo fibroblast cells derived from 10-day-old embryonated eggs (SPAFAS, Inc.) and purified as described (Snyder et al (1988a) "Avian Dis.", 32:527-534). The Mabs against various strains of IBDV were produced and characterized using protocols previously outlined (Snyder et al (1988a) "Avian Dis." 32:527-534; Snyder et al (1988b) "Avian Dis.", 32:535-539). Mabs B69 and R63 were prepared against D78 strain, whereas Mabs 8, 10, 57 and 179 were prepared against GLS strain. In addition, a new Mab 67 was prepared which was neutralizing and specific for the E/Del strain. Identification of IBDV antigens by modified antigen capture ELISA (AC-ELISA) was carried out as described (Snyder et al (1992) "Arch. Virol.", 127:89-101).

Various strains of IBDV were characterized by their reactivities with a panel of neutralizing Nabs, as shown in Table 1.

TABLE 1
Antigenic characterization of various IBDV strains by their reactivities with a panel of neutralizing MAbs

Virus Strains	Classification	Reactivities with MAbs						
		B69	R63	179	8	10	57	67
D78	Classic	+	+	+	+	+	-	-
PRG98	Classic	-	+	+	+	+	-	-
STC	Classic	+	+	+	+	+	-	-
52/70	Classic	+	+	+	+	-	-	-
OH (serotype 2)	Classic	+	+	+	+	-	-	-
E/Del	Variant	-	+	+	+	-	-	+
GLS	Variant	-	-	+	+	+	+	-
DS326	Variant	-	-	-	+	+	+	-

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All standard serotype 1 viruses reacted with Mabs B69, R63, 179 and 8, except PBG98 (a British vaccine strain, Intervet, U. K.) which did not react with Mab B69. In contrast, all the U.S. variant viruses lack the virus-neutralizing B69 epitope. In addition, GLS and DS326 variants lack an R63 epitope but share a common epitope defined by the Mab 57. Thus, on the basis of the reactivities with various Mabs, these viruses were antigenically grouped as classic, GLS, DS326 and E/Del variants.

Complementary DNA clones, containing the entire coding region of the large RNA segment of various IBDV strains, were prepared using standard cloning procedures and methods previously described (Vakharia et al (1992) "Avian Dis.", 36:736-742; Vakharia et al (1993) "J. Gen. Virol.", 74:1201-1206). The complete nucleotide sequence of these cDNA clones was determined by the dideoxy method using a Sequenase DNA sequencing kit (U.S. Biochem. Corp., Columbus, OH). DNA sequences and deduced amino acid sequences were analyzed by a PC/GENE software package (Intelligenetics, Inc.). These are reflected in Figures 5 and 6. The nucleotide sequence data of the GLS strain has been deposited with GenBank Data Libraries and has been assigned an accession number M97346.

Comparisons of the nucleotide sequence of GLS strain (3230 bp long) with eight serotype 1 and one serotype 2 IBDV strains exhibit $\geq 92\%$ and $\geq 82\%$ sequence homology, respectively; indicating that these viruses are closely related. It is interesting to find that there are only six to nine base substitutions between D78, PBG98, and Cul strains which corresponds to a difference of about 0.2% to 0.3% (results not shown). Figure 3 and Table 2 show a comparison of the deduced amino acid sequences and percent homology of the large ORF of segment A of the ten IBDV strains, including four IBDV strains used in this study.

TABLE 2
Percent amino acid sequence homology of large ORF of segment A of ten IBDV strains

Strain	GLS	DS326	E/Del	D78	Cu-1	PBG98	52/70	STC	002-73	OH
GLS										
DS326	98.7									
E/Del	98.4	98.3								
D78	98.5	98.1	97.9							
Cu-1	98.6	98.2	98.0	99.6						
PBG98	98.5	98.1	97.9	99.5	99.5					
52/70	98.1	98.1	97.9	98.4	98.5	98.3				
STC	97.7	98.0	97.5	98.4	98.5	98.3	98.3			
002-73	97.0	97.1	96.7	97.6	97.7	97.6	97.3	97.4		
OH	90.0	90.0	89.7	90.2	90.3	90.2	89.8	90.3	90.1	

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These comparisons show that the proteins are highly conserved. The degree of difference in the amino acid sequence ranges from 0.4% for the D78 versus Cu-1 comparison and 10.3% for the serotype 1 (E/Del) versus serotype 2 (OH) comparison (Table 2).

In Figure 3, alignments of the deduced amino acid sequences of the large ORF (1012 residues) of ten IBDV strains (including four used in this study) show that most of the amino acid changes occur in the central variable region between residues 213 and 332 of VP2 protein, as shown earlier by Bayliss et al (1990) "J. Gen. Virol. M, 71:1303-1312. It is interesting to note that all the U.S. variants (GLS, DS326 and E/Del) differ from the other strains in the two hydrophilic regions which are overlined in Figure 3 (residues 212 to 223 and residues 314 to 324). These two hydrophilic regions have been shown to be important in the binding of neutralizing Mabs and hence may be involved in the formation of a virus-neutralizing epitope (Heine et al (1991) "J. Gen. Virol.", 22:1835-1843). Recently, we demonstrated that the conformation dependent Mabs B69, R63, 8, 179, 10, and 57 (see Table 2) immunoprecipitate VP2 protein (Snyder et al (1992) "Arch. Virol.", 127:89-101). In addition, E/Del specific Mab 67 also binds to VP2 protein. Therefore, to identify the amino acids involved in the formation of virus-neutralizing epitopes, and hence the antigenic variation, we compared the amino acid sequences of VP2 protein of classic and variant viruses.

Comparison of the D78 sequence with the PBG98 sequence shows only four amino acid substitutions at positions 76, 249, 280 and 326. However, STC and 52/70 strains also differ from the D78 sequence at positions 76, 280 and 326 but these viruses do bind to Mab B69. This implies that Gln at position 249 (Gln249) may be involved in the binding with Mab B69. It should be noted that all U.S. variant viruses have a Gln-Lys substitution at this position and hence escape the binding

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with neutralizing Mab B69. Similarly, comparison of the GLS sequence with the DS326 sequence in the variable region shows six amino acid substitutions at positions 222, 253, 269, 274, 311 and 320. However, other strains of IBDV that do bind to Mab 179 have amino acid substitutions at positions 222, 253, 269 and 274 that are conservative in nature. Therefore, this suggests that Glu311 and Gln320 may be involved in the binding with Mab 179. Again, comparison of GLS and DS326 sequences with all other IBDV sequences shows a unique Ala→Glu substitution at position 321, suggesting the contribution of this residue in the binding with Mab 57. Since Mab 57 does not compete with Mab R63, it is conceivable that Ala321 may contribute to the binding with Mab R63. Similarly, comparison of E/Del sequence with other sequences shows five unique substitutions at positions 213, 286, 309, 318 and 323. However, comparison of this E/Del sequence (from tissue culture derived virus) with previously published VP2 A/Del and E/Del sequences (bursa derived virus) suggests the involvement of Ile286, Asp318 and Glu323 in the binding with Mab 67 since residues at positions 213 and 309 are not substituted in A/Del and E/Del sequences, respectively (Heine et al (1991) "J. Gen. Virol.", 22:1835-1843; Lana et al (1992) "Virus Genes", 6:247-259; Vakharia et al (1992) "Avian Dis.", 36:736-742).

Comparisons of the amino acid sequence also show a striking difference between serotype 1 and serotype 2 sequences. In serotype 2 OH strain, there is an insertion of an amino acid residue at position 249 (serine) and a deletion of a residue at position 680. Previously, it has been shown that serotype 2 viruses are naturally avirulent and do not cause any pathological lesions in chickens (Ismail et al (1988) "Avian Dis.", 32:757-759). Thus, these subtle changes in the structural proteins of serotype 2 OH strain may play an important role in the pathogenicity of the virus. Moreover, it has been hypothesized that an amino acid sequence motif, S-W-S-A-S-G-S, (residues 326 to 332) is conserved only in

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virulent strains and could be involved in virulence (Heine et al (1991) "J. Gen. Virol.", 22:1835-1843). This sequence motif was also conserved in various pathogenic strains of IBDV isolated in Japan (Lin et al (1993) "Avian Dis.", 37:315-323). comparison of the amino acid sequences in this heptapeptide region reveals that nonpathogenic serotype 2 OH strain has three substitutions, whereas mildly pathogenic strains of serotype 1 (D78, Cu-1, PBG98 and 002-73) have one or two substitutions in this region. Moreover, comparison of the hydrophilicity plots of the variable region (amino acids 213 to 332) of variant serotype 1 strains and serotype 2 OH strain indicates a drastic reduction in the second hydrophilic peak region (amino acid residues 314 to 324) for serotype 2 (results not shown). Since most of the amino acid residues causing antigenic variation reside in this region, these residues may play an important role in the formation of virus-neutralizing epitopes, as well as serotype specificity.

To evaluate the antigenic relatedness of structural proteins of various IBDV strains, a phylogenetic tree was constructed, based on the large ORF sequences of ten IBDV strains, including the U.S. variant strains examined in this study (Figure 4). Three distinguishable lineages were formed. The first one, which is most distant from the others, is serotype 2 OH strain, and the second one is the geographically distant Australian serotype 1 strain (002-73). The third lineage consists of four distinct groups. The first and second group include highly pathogenic strains, namely, standard challenge (STC) strain from U. S. and the British field strain (52/70). The third group comprises all the European strains: the vaccine strains D78 (Holland), PBG98 (U.K.), and mildly pathogenic strain Cu-1 (Germany). The fourth group consists of the U.S. variant strains in which E/Del forms a different subgroup. The groups formed by the phylogenetic analysis correlate very well with the Mabs reactivity patterns (see Table 1). As shown in Figure 4, all

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36:736-742). To insert the chimeric IBDV structural genes in the *Baculovirus* genome, plasmid pB69GLS was completely digested with *Bst*EII enzyme and partially with the *Bam*HI enzyme, combined with the *Nhe*I-*Bst*EII fragment (derived from plasmid pGLSBacI, see Vakharia et al (1993) "J. Gen. Virol.", 74:1201-1206) and then ligated to the *Nhe*I-*Bam*HI cut transfer vector pBlueBacII (Invitrogen Corp., San Diego, CA). Finally, recombinant *baculovirus* I-7 was obtained using previously described procedures (Vakharia et al (1993) "J. Gen. Virol.", 74:1201-1206). See Table 3.

Preparation of an inoculum for immunization

Spodoptera frugiperda SF9 cells, infected at a multiplicity of 5 PFU per cell with the I-7 recombinant *baculovirus*, were propagated as suspension cultures in one liter flasks containing Hink's TNM-FH medium (JHR Biosciences, Lenexa, KS) supplemented with 10% fetal calf serum at 28°C for 3 to 4 days. The infected cells were recovered by low speed centrifugation, washed two times with PBS, and resuspended in a minimum volume of PBS. The cell slurry was sonicated on and ice bath three times for 1 min, with 2 min intervals and clarified by low speed centrifugation. An aliquot of each cell lysate was tested with anti-IBDV Mabs by AC-ELISA to estimate the antigenic mass present (Snyder et al (1998b) "Avian Dis.", 32:535-539). Preparations having the highest antigenic mass were pooled and comparatively titrated in AC-ELISA against the V-IBDV-7-1 recombinant *baculovirus* IBDV vaccine used in a previous study (Vakharia et al (1993) "J. Gen. Virol.", 74:1201-1206). The antigenic mass of the I-7 recombinant preparation, as determined by AC-ELISA with group specific neutralizing Mab 8, was adjusted by dilution to be the same as the V-IBDV-7-1 vaccine, and then it was emulsified with an equal volume of Freund's incomplete adjuvant and used for inoculation.

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the U.S. variant viruses which lack the B69 epitope form a distinct group, whereas all the classic viruses containing a B69 epitope form another group (except PBG98). In addition, closely related GLS and DS326 strains containing a common Mab 57 epitope and lacking an R63 epitope could be separated from the other variant E/Del strain.

Based on this information, a recombinant vaccine was constructed as follows:

Construction of recombinant baculovirus clones containing chimeric IBDV genes

A recombinant baculovirus which expresses a chimeric VP2, VP3 and VP4 structural proteins of the GLS strain was constructed and assessed. The recombinant baculovirus expressed a chimeric VP2 protein incorporating all Mab defined GLS neutralization sites, as well as one neutralization site (B69) which is specific for Classic strains of IBDV in the form of a VP2-VP4-VP3 segment.

Complementary DNA clones, containing the entire coding region of the large RNA segment of the GLS and D78 IBDV strains, were prepared using standard cloning procedures and methods previously described (Vakharia et al (1992) "Avian Dis.", 36:736-742; Vakharia et al (1993) "J. Gen. Virol.", 74:1201-1206). To insert the gene sequence encoding the B69 epitope of the D78 IBDV strain, plasmid pB69GLS was constructed as follows (see Figure 1). Full-length cDNA clones of D78 and GLS (plasmids pD78 and pGLS-5) were digested with *NdeI*-*NarI* and *NarI*-*SpeI* enzymes to release a *NdeI*-*NarI* (0.26 kb) and a *NarI*-*SpeI* (0.28 kb) fragments, respectively. These two fragments were then ligated into the *NdeI*-*SpeI* cut plasmid pGLS-5 to obtain a chimeric plasmid pB69GLS. As a result of this insertion, three amino acids were substituted in the GLS VP2 protein. These substitutions were at positions 222 (Thr-Pro), 249 (Lys-Gln) and 254 (Ser-Gly) in the variable region of the VP2 protein (Vakharia et al (1992) "Avian Dis.",

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Viruses

The challenge viruses: Classic strains IM and STC, and variant strains E/Del and GLS-5 were obtained from previously acknowledged sources (Snyder et al (1988a) "Avian Dis.", 32:527-534; Snyder et al (1992) "Arch. Virol.", 127:89-101). After intraocular instillation, challenge viruses were titrated in the bursae of specific-pathogen-free (SPF) chickens (SPAFAS, Inc., Storrs, Conn.). For strains STC, E/Del and GLS-5, a 100 chick infective fifty percent dose (100 CID₅₀) was determined based on bursa to body weight measurements. One hundred lethal doses (100 LD) of the IM strain were calculated based on mortality at 8 days post-inoculation (PI).

Chicken inoculations and IBDV challenge

White leghorn SPF chickens were hatched and reared in HEPA filtered isolation units (Monair Anderson, Peachtree City, GA). Eight-week old chickens were prebled, individually wing banded, divided among 10 groups of 15 chicks each and treated as follows. Chickens of groups I-V received no inoculations and served as either negative or positive challenge controls. Chickens of group V-X were inoculated intramuscularly with 0.5 ml of the 1-7 inoculum prepared above from recombinant Baculovirus infected cell lysates. At 3 weeks PI, all chickens were bled and chickens of groups II-IX were challenged with the appropriate IBDV challenge strain by ocular instillation. Four days post-challenge, 5 chickens from each group were humanely sacrificed and their cloacal bursa were removed. Each bursa was processed and subsequently evaluated for the presence of IBDV antigen by AC-ELISA as described (Snyder et al (1998b) "Avian Dis.", 32:535-539). In addition, chickens in the IM challenged groups were scored as dead, and humanely sacrificed when they became obviously moribund due to IM challenge. Eight days post-infection, the remaining chickens in all groups were sacrificed and weighed.

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The bursa of *Fabricius* from each chicken was carefully excised and also weighed. Bursa weight to body weight ratio was calculated for each chicken as described by Lucio and Hitchner (Lucio et al (1979) "Avian Dis.", 23:466-478). Any value for individually challenged chickens falling plus or minus two standard deviation units from the mean of the corresponding control group was scored as a positive indicator of IBDV infection. Opened bursae were fixed by immersion in 10% neutral buffered formalin. Transverse portions of bursae were processed through graded alcohols and xylene, embedded into paraffin, sectioned, stained with hematoxylin-eosin, and examined with a light microscope. Protection against challenge was defined as the absence of any IBDV-induced lesions in the bursa of *Fabricius*.

Serological evaluation

The Classic D78 strain, as well as the cell culture adapted variant GLS strain of IBDV were grown in primary chicken embryo fibroblast cells and used in virus neutralization (VN) tests to test sera from the vaccine trial essentially as described (Snyder et al (1988a) "Avian Dis.", 32:527-534). Serum from the trials was also tested for the presence of anti-IBDV antibody using a commercially available IBDV antibody ELISA kit (Kirkegaard and Perry, Gaithersburg, MD).

Evaluation of vaccines and challenge viruses

The antigenic content of the I-7 GLS chimeric IBDV vaccine was assessed in AC-ELISA with a panel of VP2 and VP3 specific Mabs. The relative antigenic mass of each epitope expressed in the I-7 vaccine was compared to previously tested lots of *Baculovirus* expressed unmodified GLS subunit vaccines (Vakharia et al (1993) "J. Gen. Virol.", 74:1201-1206). The status of each Mab defined epitope on the I-7 chimeric vaccine was also compared to the status of those Mab defined epitopes

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occurring on wild type IBDV challenge viruses used to evaluate the efficacy of the I-7 vaccine. Table 3 shows that antigenic mass levels at the 8, 57, and B29 epitopes for the current I-7 chimeric vaccine were similar to a recently tested unmodified V-IBDV-7-1 GLS subunit vaccine, but approximately 4-fold higher than the original unmodified V-IBDV-7 vaccine.

TABLE 3

Comparative levels of IBDV, VP2, and VP3 monoclonal antibody (Mab) defined epitopes of recombinant baculovirus expressing IBDV proteins and status of Mab defined epitopes on challenge viruses used.

	Relative level of Mab epitope ^a					Challenge	Status of Mab epitope ^a			
	8	57	B69	67	B29		8 ^c	57 ^c	B69 ^c	67 ^c
Vaccine						Virus				B29 ^b
V-IBD-7 ^s	1	1	0	0	1	GLS	+	+	-	-
V-IBD-7-1 ^s	3	3	0	0	2	STC	+	-	+	-
I-7 ^r	3	3	9	0	2	IM	+	-	+	-
						E/Del	+	-	-	+

^a The relative level of each Mab epitope was determined by AC-ELISA, and the level of each Mab epitope was set to 1 for the V-IBD-7 vaccine previously used (15). Maximum level is 9. Each 1.0 increment represents approximately twice the amount of the epitope present in the original V-IBD-7 vaccine. The V-IBD-7-1 vaccine was also previously reported (16).

^b The status of Mab epitopes was determined by AC-ELISA and is presented as present (+) or absent (-).

^c Neutralizing Mab epitope resides on VP2 of IBDV.

^d Non-neutralizing Mab epitope resides on VP3 or IBDV.

^e Recombinant baculovirus vaccines incorporating unmodified large segment A GLS proteins.

^f Current recombinant baculovirus vaccine incorporating modified chimeric large segment A GLS proteins.

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A major difference in the unmodified and chimeric vaccines was the appearance of the classic B69 epitope in the chimeric GLS product. The level of the B69 epitope was arbitrarily set at 9 since no comparisons could be made to the unmodified GLS subunit vaccines. By comparing the status of Mab defined epitopes on the challenge viruses with the unmodified and chimeric GLS subunit vaccines (Table 3), it could be seen that while the chimeric product had expressed the B69 epitope found on the Classic STC and IM challenge viruses, that it also retained all of the homologous GLS epitopes.

Active-cross protection

Table 4 shows the results of a cross-protection trial and serological results obtained prior to challenge.

TABLE 4

Active cross-protection induced 2-weeks post immunization with baculovirus expressed chimeric I-7 IBDV antigens and associated prechallenge serology.

Group No.	Vaccination ^a	Challenge ^b	Number Protected			Mean VN Titer ³ Log		Mean ELISA
			AC-ELISA ^c	Histo ^d	BBWR ^e	D78	GLS	
I	None	None	N/A	NA	NA	≤4	≤4	0
II	None	STC	0/5	0/10	0/10	≤4	≤4	0
III	None	IM	0/5	0/5 ^b	5/5 ^b	≤4	≤4	0
IV	None	E/Del	0/5	0/10	0/10	≤4	≤4	0
V	None	GLS-5	0/5	0/10	0/10	≤4	≤4	0
VI	I-7	STC	5/5	10/10	10/10	107.7(1.8) ^f	10.4(1.4) ^f	1235(312) ^f
VII	I-7	IM	5/5	10/10	10/10	10.0(1.4)	10.4(2.1)	1201(791)
VIII	I-7	E/Del	5/5	10/10	10/10	11.4(1.2)	10.6(1.9)	1089(409)
IX	I-7	GLS-5	5/5	10/10	10/10	11.0(1.5)	12.0(2.0)	1220(339)
X	I-7	None	5/5	NA	NA	9.9(1.4)	9.3(1.4)	1140(473)

^aVaccination was given at 8-weeks of age.

^bChallenge virus was given by intraocular instillation 3-weeks post immunization or at 11-weeks of age for controls.

^cProtection was determined by AC-ELISA examination of 1/3 of each group 4-days post-challenge.

^dProtection was determined histologically and by bursa to body weight ratios at 8-days.

^eFive chickens were scored as dead due to IM challenge prior to 8-days post-challenge.

^f One standard deviation.

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Groups II - V served as challenge controls and as indicated by AC-ELISA, bursa to body weight and histological assessments, all non-vaccinated chickens were fully susceptible to virulent IBDV challenge with all strains used. The IM challenge produced lethal disease in one-third of the control group chicks. In contrast, 8-week old chickens comprising Groups VI - IX were vaccinated once with the GLS chimeric vaccine, and 3-weeks PI all vaccinated chickens were completely protected from challenge by all challenge viruses, including lethal disease produced in controls by the IM strain. Serologically, titers from reciprocal-cross VN tests conducted on prechallenge sera with the D78 and GLS tissue culture viruses were essentially within 2-fold of one another. Mean ELISA titers were relatively low, but were also uniform between the vaccinated groups.

Characterization of vaccines

In initial studies with *Baculovirus* expressed subunit GLS vaccines, after administration of two doses, the V-IBDV-7 GLS vaccine (Table 3) could only induce active antibody levels capable of providing 79% protection against homologous GLS challenge (Vakharia et al (1993) "J. Gen. Virol.", 74:1201-1206). In a subsequent study, the antigenic mass of the original V-IBDV-7 vaccine was increased approximately 4-fold (calculated at the group specific Mab 8 site) and initiated one dose and two dose vaccination cross-challenge trials with the unmodified GLS subunit vaccine designated as V-IBDV-7-1 (Table 3). In those trials, two doses of the vaccine yielded complete cross-protection against virulent STC, E/DEL and GLS challenge. However, in the one vaccine dose trial, while complete protection was attained against challenge with variant E/DEL and GLS viruses, only 44% protection was achieved against the more distantly related Classic STC virus. Those studies could mean that simply by increasing the antigenic mass and/or doses of the vaccine that better cross-protection could be obtained. However, it was also evident in the absence of homologous vaccination that lower levels of

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antibody, induced by one dose of the GLS V-IBDV-7-1 subunit vaccine, were not sufficiently cross-protective against Classic IBDV challenge. This could mean that in even lower levels of antibody, such as in cases of waning maternal antibody, that cross-protection would likely be even more reduced. Indeed, although not challenged with the STC virus, in some passive maternal antibody studies conducted using another dosage of the V-IBDV-7 vaccine, while homologous GLS protection was afforded, progeny of vaccinated hens were only 57% protected against a more closely related E/DEL challenge.

In a single-dose vaccination cross-challenge trial, the chimeric GLS I-7 vaccine, which incorporated the Classic B69 neutralization epitope, was evaluated. In order to make the current trial comparable to previous trials, the I-7 vaccine was formulated such that by AC-ELISA the relative antigenic mass of the I-7 chimeric subunit vaccine was nearly identical to the unmodified V-IBDV-7-1 vaccine previously used (Table 3). Table 4 shows the results of the cross-challenge after a single dose of the I-7 vaccine was administered. Results were similar to those obtained with the unmodified V-IBDV-7-1 vaccine previously used in that protection against the GLS and E/DEL strains was complete. However, the I-7 vaccine yielded complete protection against pathogenic and lethal challenge by the Classic STC and IM strains respectively. Since the antigenic mass of the GLS and group common epitopes on V-IBDV-7 and I-7 vaccines were carefully equilibrated and equal, it is reasonable to conclude that the comparative increase in efficacy of the I-7 vaccine against challenge with Classic IBDV strains was due solely to the incorporation of the Classic IBDV B69 neutralization epitope in the GLS VP2 protein sequence.

VIRUS-LIKE PARTICLES

As noted above, the recombinant cDNA and immunogens expressed thereby, of this invention may be confined to the VP2 immunogenic region. In other words, it may be sufficient to prepare a cDNA clone encoding epitopic determinants for a

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base IBDV, e.g., GLS, as well as a second IBDV epitopic determinant, such as D78. Other epitopic determinants, all in the VP2 epitopic determinant region may be incorporated, cloned and expressed as discussed above.

As reflected in Figure 2, virus-like particles are generated by the expression of DNA encoding the VP2-VP4-VP3 structural protein sequences. These virus-like particle immunogens can be separated from the corresponding VP2 only immunogens, both in terms of monoclonal antibody and by conventional separation measures, such as electrophoresis and chromatography. The difference in reactivity with monoclonal antibody strongly indicates, however, that epitopes present in the VP2-VP4-VP3 structural protein sequence-induced virus-like particles are present that are not present in immunogens expressed by the identical VP2 only region. These epitopes are "both linear and conformational" epitopes. Conformational epitopes differ from linear epitopes and are reflected in the conformation, not only in amino acid sequence of the actual virus. As a result, inoculation of poultry with a recombinant virus-like particle may provide even superior protection against field challenge from IBDV than inoculation with the immunogens of the VP2 region only. This is due to the spontaneous assembly of all the structural elements of the virus.

Applicants have discovered that the expression of the VP2 region as part of the VP2-VP4-VP3 structural protein single segment generates virus-like particles such as those of Figure 2. These particles have been demonstrated to react with antibodies which do not react similarly with the identical recombinant VP2 immunogen. Thus, the virus-like particles may give rise to higher antibody titers, and/or subtly different (broader) protection when a poultry host is inoculated therewith.

The invention herein therefore embraces (1) recombinant VP2 immunogens comprising epitopic determinants of at least two different IBDV strains and (2) virus-like particles of VP2-VP4-VP3 segments wherein the VP2 region again comprises

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epitopic determinants of at least two different IBDV strain, as well as the nucleotide sequences encoding both 1 and 2, and vaccines embracing the same.

RECOMBINANT EPITOPIC DETERMINANT COMBINATIONS:

As reflected in the examples set forth above, genetic epitopic determinants for an IBDV strain can be inserted in the VP2 region of a different, base IBDV genetic sequence, and subsequently used to express an immunogen exhibiting epitopes for both IBDV. Indeed, the examples above demonstrate the combination of at least three different IBDV epitopic determinants. More can be combined. The resulting vaccine includes an active agent, the expressed immunogen, which provides challenge protection against a broad spectrum of IBDV, rather than prior art virus-based vaccines which give protection against a single strain, or a single family of strains.

Figure 7 reflects the amino acid identities for the epitopic determinant region for seven different IBDV. These are not intended to be limiting, but are representative. Desirable recombinant immunogens, both VP2 only and virus-like particle VP2-VP4-VP3 immunogens are made by substituting the genetic epitopic determinants for the varying amino acids at the identified locations in Figure 7 (locations not identified are conserved throughout the IBDV strains). This induces the expression of the inventive immunogens. Clearly, the possible combinations, while large in number, are limited, and may be investigated with routine skill. Representative combinations will tend to reflect combinations of epitopic determinants for dominant IBDV.

A E/Del/GLS recombinant may include changes in the E/Del epitopic determinant region at position 213, Asn-Asp, 253 Gln-His and 169 Thr Ser.

A DS326/D78 recombinant may include the amino acid, and corresponding nucleotide substitutions at 76Ser-Gly, 249 Lys-Gln, 253 Gln-His and 270 Ala-Thr substitutions.

Obviously, a wide variety of combinations are possible

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and will occur to those of skill in the art. The epitopic determinant region, roughly including the region from amino acid 5-433 of the VP2 region, thus constitutes a recombinant "cassette" which may be tailored by site-specific mutagenesis to achieve amino acid insertion and/or deletion to provide desired recombinant cDNA clones, polypeptides, virus-like particles and vaccines with improved protection against a wide variety of IBDV.

LETHAL IBDV, MONOCLONAL ANTIBODY AND VACCINE THEREFORE

As noted, typically, IBDV infection creates an immunosuppressive condition, and is reflected in lesions in the bursa of *Fabricius*. This is typical of IBDV countered in the United States. There exist, however, lethal IBDV, that is, IBDV infections which results in chicken mortality directly as a result of IBDV infection. While vaccines have been developed on the basis of isolation of these IBDV, the resulting vaccines are "hot", that is, they themselves create or induce an immunosuppressive condition, and the inoculated chick must be bolstered with antibodies to other infectious agents. This method of protection is so undesirable as to have been discontinued in most commercial poultry houses in Europe. No adequate safe vaccine against the lethal IBDV is currently available.

The inventors have developed a monoclonal antibody, Mab 21, deposited under Budapest Treaty conditions at the American Type Culture Collection, Deposit Accession No. ATCC HB 11566. This monoclonal antibody is specific and neutralizing for lethal IBDV strains. The specificity is reflected in Table 5, which confirms that unlike other monoclonal antibody, Mab 21 is specific for an epitope exhibited only by IBDV strains having lethal potential.

TABLE 5

Source	IBOV Strain Lethal Potential	Comment	B29	8	179	10	63	69	21	67	57	50
Sharma	IM+		+	+	+	+	+	+	+	-	-	-
USDA	IM		+	+	+	+	+	+	+	-	-	-
Spafas	STC		+	+	+	+	+	+	+	-	-	-
Edgar	2512 (Winterfield)		+	+	+	+	+	+	+	-	-	-
	Edgar	(vaccine hot)	+	+	+	+	+	+	+	-	-	-
Sterwin	Pathogenic Virus											
	Bursa Vac	(vaccine hot)	+	+	+	+	+	+	+	-	-	-
	Vaccine Virus											
ASL	Univax-8D	(ST 14)	+	+	+	+	+	+	+	-	-	-
Select	Bursal Disease Vaccine	(Luk)	+	+	+	+	+	+	+	-	-	-
Select		(STD + VAR)	+	+	+	+	+	+	+	-	-	-
Key Vet		(D78)	+	+	+	+	+	+	+	-	-	-
Key Vet	Bio-Burs I	(Luk)	+	+	+	+	+	+	+	-	-	-
Key Vet	Bio-Burs W	(D78)	+	+	+	+	+	+	+	-	-	-
Key Vet	Key-Burs	(Luk)	+	+	+	+	+	+	+	-	-	-
MBL	Maryland	(Master seed)	+	+	+	+	+	+	+	-	-	-
Sterwin	BVM	(Basendate)	+	+	+	+	+	+	+	-	-	-
Sterwin	1048-E		+	+	+	+	+	+	+	-	-	-
Lukert	BVM	(Lab Strain)	+	+	+	+	+	+	+	-	-	-
CEVA	Bursa Blend	(2512)	+	+	+	+	+	+	+	-	-	-
InterVet	D78		+	+	+	+	+	+	+	-	-	-
InterVet	Prime Vac		+	+	+	+	+	+	+	+	+	+
InterVet	8903		+	+	+	+	+	+	+	+	+	+
Solvay	Bursine	(Luk)	+	+	+	+	+	+	+	-	-	-
Solvay	Bursine II	(Luk+)	+	+	+	+	+	+	+	-	-	-
	Lab Virus											
JKR	E/Del		+	+	+	+	+	+	+	+	+	+
JKR	A/Del		+	+	+	+	+	+	+	+	+	+
KKR	D/Del		+	+	+	+	+	+	+	+	+	+
DBS	GLS		+	+	+	+	+	+	+	+	+	+
DBS	DS326		+	+	+	+	+	+	+	+	+	+
*skeels	S977	(Serotype II)	+	+	+	+	+	+	+	+	+	+
OH			+	+	+	+	+	+	+	+	+	+

* Field Strains: All classic field strains tested to date which were isolated in the U.S. have the 21 marker
 NOTE: 1. Lukert and STC are Edgar derivatives. 2. Univax is a Bursa Vac derivative. 3. Bursa Blend is a 2512 Winterfield derivative.

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It should be noted that throughout this application, reference is made to a variety of monoclonal antibody which are used to confirm the presence of epitopes of different IBDV in the inventive recombinant chimeric immunogens of the application. These monoclonal antibody have also been deposited under Budapest Treaty conditions and are freely available. They are not, however, necessary for the practice of this invention, and do not constitute an aspect thereof. This should be contrasted with Mab 21.

Like other Mab developed by the inventors herein for IBDV, passive immunization against IBDV lethal strains, particularly designed to achieve immunization in a uniform, standardized level, and to augment any maternally derived levels against lethal IBDV field infection can be obtained by vaccinating one-day old chicks with a vaccine comprising a pharmacologically acceptable carrier such as those described above, in which is present an amount of Mab 21 effective to provide enhanced protection for the inoculated chicks.

The necessary level of protection can be conferred to by a single dose of the vaccine administered *in ova* or to a day-old chick having a Mab 21 concentration of between 1 microgram and 1 milligram, or repeated vaccinations having a smaller effective dose, but carried out over time. If repeated vaccinations are used, the dosage levels should range between 1 microgram and 1 milligram. The concentration level needed to vaccinate older chickens increases with the weight of the bird and can be determined empirically.

Further investigation of the amino acid sequences of the lethal strains in the epitopic determinant region reflects the highly conserved 279 identity Asn at position 279 of VP2, in non-lethal strains, with a conserved Asp identity at the same position in lethal strains. Accordingly, the lethal strain epitopic determinant recognized by Mab 21, unique to the lethal strains, empirically differs from non-lethal IBDV by the substitution 279 Asp-Asn. According to the methods set forth above, a chimeric, recombinant immunogen conferring effective protection against lethal IBDV, something not

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possible previously with any type of vaccine without inducing an immunosuppressive condition, may be prepared by inserting the genetic epitopic determinant for 279 Asp in a non-lethal base IBDV, such as GLS. This will confer protection against the base IBDV, the lethal IBDV, as well as all other IBDV whose genetic epitopic determinants are inserted. Vaccines prepared from these immunogens, whether VP2 only, or in the form of virus-like particles of VP2-VP-VP3 segments, are used in the same fashion as discussed above.

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Claims:

1. A chimeric polypeptide immunogen comprising the VP2 amino acid sequence of a first infectious bursal disease virus (IBDV) except for at least one amino acid X, wherein X is an epitopic determinant from a second IBDV strain.
2. The immunogen of Claim 1, wherein said VP2 amino acid sequence comprises a plurality of a different epitopic determinant X.
3. The immunogen of Claim 2, wherein said plurality of epitopic determinants X are from at least two different IBDV strains.
4. The immunogen of Claim 1, wherein said IBDV strains are selected from the group consisting of GLS, E/Del, D78, DS326, RS593, Cu-1, PBG98, 52/70, STC and 002-73.
5. The immunogen of Claim 1, wherein said immunogen comprises the amino acid sequence, in order, for IBDV structural proteins VP2-VP4-VP3.
6. The immunogen of Claim 5, wherein said immunogen is in the form of a virus-like particle.
7. The immunogen of Claim 6, wherein said immunogen exhibits at least one IBDV conformational epitope.
8. The immunogen of Claim 1, wherein said amino acid sequence includes an epitopic determinant X of a lethal IBDV strain.
9. The immunogen of Claim 8, wherein said epitopic determinant of lethal IBDV strains comprises the amino acid Asp at position 279 of the VP2 sequence.

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10. A preparation sufficient to provide poultry inoculated therewith resistance to IBDV challenge from at least two different IBDV strains, comprising, as an active agent, an effective amount of the immunogen of any one of Claims 1-9, and a pharmacologically acceptable carrier.

11. An avirulent immunogen which confirms on poultry inoculated therewith protection against challenge from IBDV lethal strains, said immunogen comprising the VP2 amino acid sequence of an IBDV, wherein position 279 of said VP2 amino acid is Asp.

12. The immunogen of Claim 11, wherein said immunogen comprises, in order, amino acid sequences for VP2-VP4-VP3 IBDV structural proteins.

13. The immunogen of Claim 12, in the form of virus-like particles.

14. A monoclonal antibody which binds, under AC-ELISA conditions, to IBDV lethal strains, and has the epitope binding characteristics of the monoclonal antibody expressed by the cell line deposited under Accession No. ATCC HB 11566.

15. The monoclonal antibody of Claim 14, wherein said monoclonal antibody is obtained, directly or indirectly, from said cell line.

16. The monoclonal antibody of Claim 15, wherein said antibody is the antibody expressed by said cell line.

17. A preparation for conferring passive immunity in a poultry inoculated therewith against IBDV lethal strain challenge, comprising, as an effective agent, the monoclonal antibody of any one of Claims 14-16 in an effective amount, and

a physiologically acceptable carrier.

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18. A chimeric cDNA which, when operably inserted as heterologous DNA in the DNA of an expression host, encodes the immunogen of any one of Claims 1-9.

19. A transfection vehicle for the infection of an expression host, comprising the cDNA of Claim 18 as operably connected in the DNA of *baculovirus* fowlpox virus, turkey herpes virus or adenovirus.

20. An expression vehicle for the expression of the immunogen of Claims 1-9, comprising an expression host selected from the group consisting of SF9 cells, chicken embryo fibroblast cells, chicken embryo kidney cells and vero cells transfected with the vehicle of Claim 19.

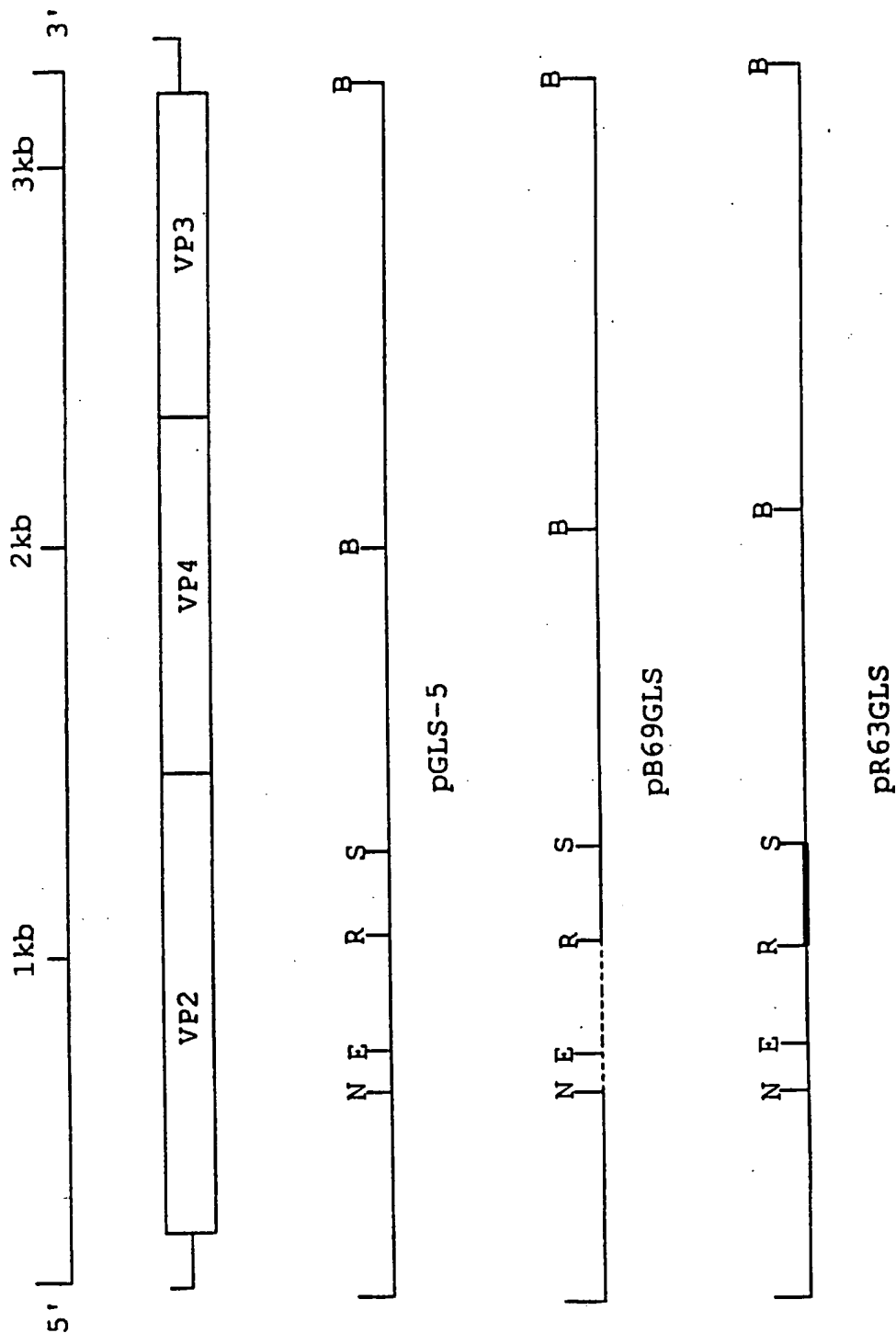
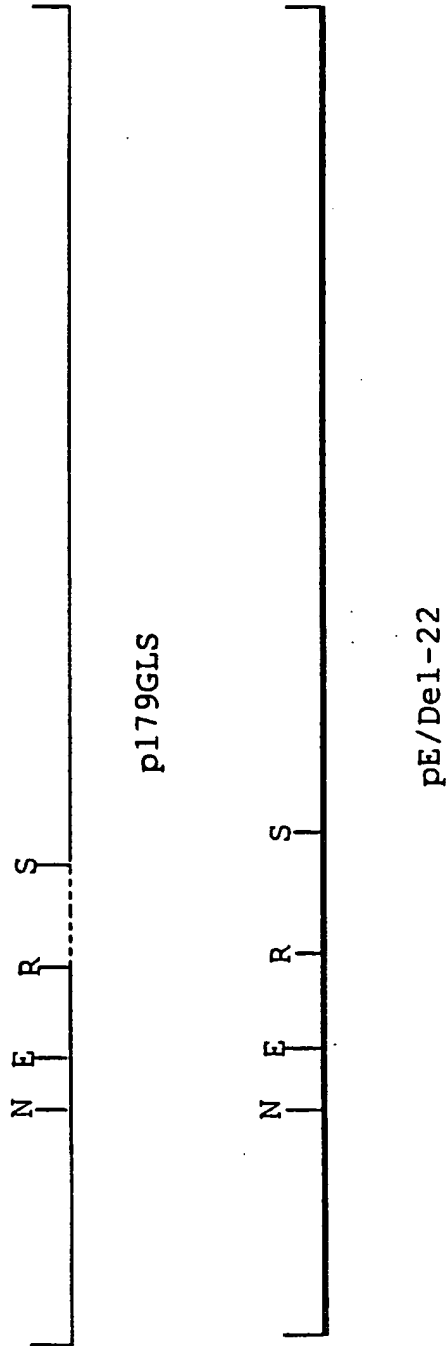


FIG. 1A

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FIG. 1B



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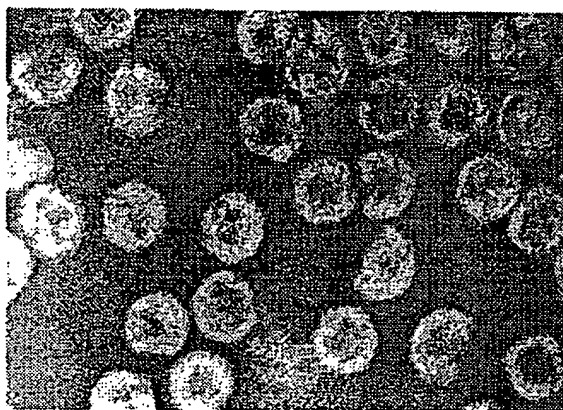


FIG.2A

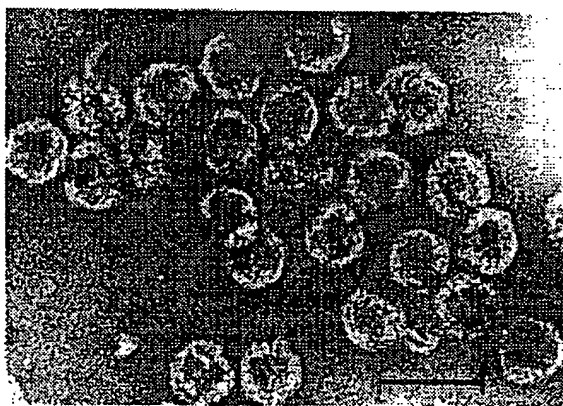


FIG.2B

FIG. 3A

	10	20	30	40	50	60
GLS	MTNLQDQTQQ	IVPFIRSLLM	PTTGPASIPD	DTLEKHTLRS	ETSTYNLTVG	DTGSGLIVFF
DS326	-----	-----	-----	-----	-----	-----
E/Del	-----H-	-----	-----	-----	-----	-----
D78	-----	-----	-----	-----	-----	-----
Cu-1	-----	-----	-----	-----	-----	-----
PBG98	XXXXXXXXXX	XXXXXXXXXX	-----	-----	-----	-----
52/70	-----	-----	-----	-----	-----	-----
STC	-----	-----	-----	-----	-----	-----
002-73	---S---	-----	-----	-----	-----	-----
OH	---M-H---	-----	-----	-----	-----	-----

70	80	90	100	110	120
PGFPGSIVGA	HYTLQSNQNY	KFDQMLLTAQ	NLPASYNQCR	LVSRSLTVRS	STLPGGVYAL
-	-	-	-	-	-
-	-	-	-	-	-
-	-S-	-	-	-	-
-	-	-	-	-	-
-	-G-	-	-	-	-
-	-	-	-	-	-
-	-	-	-	-	-
-	-	-	-	-	-
-	-	-	-	-	-
-	-	-	-	-	-
-	-L-	-	-	-	-
-	-	-	-	-	-
-	-M-	-	-	-	-
-	-	-	-	-	-
-V-	-S-Q-	-	-V-	-	-

FIG. 3B

GLS	10	20	30	40	50	60
DS326	NGTINAVTFQ	GSLSELTQVS	YNGLSMTAN	INDKIGNVLV	GEGVTLSLP	TSYDLGYVRL
E/DeI	-	-	-	-	-	-
D78	-	-	-	-	-	-
Cu-1	-	-	-	-	-	-
PBG98	-	-	-	-	-	-
52/70	-	-	-	-	-	-
STC	-	-	-	-	-	-
002-73	-	-	-	-	-	-
OH	-	-Y-	-	-	-	-S-

70	80	90	100	110	
GDPIPAIGLD	PKMVATCDSS	DRPRVYTITA	ADDYQFSSQY	QTGGVTITLF	SANIDAITSL - 240
-	-	-	-	-S-	-
-	-	-	-N-	-	-
-	-	-	-	-P-	-
-	-	-	-	-P-	-
-	-	-	-	-P-	-
-	-	-	-	-P-	-
-	-	-	-	-P-	-
-	-	-	-	-P-	-
-	-	-	-	-P-	-N-
-A-	-LM-	-V-	-E-	IPS-KT-	T-L-

FIG. 3C

GLS	10	20	30	40	50	60
DS326	SVGGELVF	KTSVHSLVLGAT	IYLGFDGSA	VITRAVAANN	GLTTGTDNLM	PFNLVIPTNE
E/Del	-----Q-----	-----T-----	-----A-----	-----A-----	-----S-----	-----S-----
D78	-----Q-----	-----T-----	-----A-----	-----A-----	-----S-----	-----S-----
Cu-1	-----Q-----	-----T-----	-----A-----	-----A-----	-----S-----	-----S-----
PBG98	-----R-----	-----T-----	-----A-----	-----A-----	-----S-----	-----S-----
52/70	-----I-----	-----Q-----	-----T-----	-----A-----	-----S-----	-----S-----
STC	-----Q-----	-----F-----	-----T-----	-----A-----	-----S-----	-----S-----
002-73	-----Q-----	-----V-----	-----T-----	-----A-----	-----S-----	-----S-----
OH	-----I-SQVTI-SIEVDV-----	-----F-----	-----TE-----	-----TVK-----	-----N-V-----	-----GG-S-----

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ITQPITSIKL	70	80	90	100	110	360
-----K-----	EGDQMSWSAS	GSLAVTIHGG	NYPGALRPVT	LVAYERVATG	-----	-----
-----I-----	-----D-----	-----R-----	-----K-----	-----R-----	-----	-----
-----V-----	-----L-----	-----R-----	-----R-----	-----R-----	-----	-----
-----M-----	-----Y-R-T-----	-----PI-----	-----TV-----	-----T-----	-----V-----	-----A-----

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FIG. 3D

	10	20	30	40	50	60
GLS	SVVTVAGVSN	FELIPNELA	KNLVTEYGRF	DPGAMNYTKL	ILSERDRLGI	KTVWPTREY
DS326	-	-	-	-	-	-
E/Del	-	-	-	-	-H-	-
D78	-	-	-	-	-	-
Cu-1	-	-	-	-	-	-
PBG98	-	-	-	-	-	-
52/70	-	-	-	-	-	-
STC	-	-	-	-	-	-
002-73	-	-	-	-	-	-
OH	-	-	-	-	-	-

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[illegible]

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FIG. 3H

GLS	10	20	30	40	50	60
DS326	EARGPTPEEA	QREKDRISK	KMETMGIYFA	TPEWVALNGH	RGPSPGQLKY	WQNTREIPDP
E/Del	-----	-----	-----	-----	-----	-----
D78	-----	-----	-----	-----	-----	-----
Cu-1	-----	-----	-----	-----	-----	-----
PBG98	-----	-----	-----	-----	-----	-----
52/70	-----	-K-----	-----	-----	-----	-----
STC	-----	-A-----	-----	-A-----	-----	-----
002-73	-----	-----	-A-----	-----	-----	-----
OH	-----	-A-A-----	-----	-----	-----	-E-----

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NEDYLDYVHA	70	80	90	100	110	960
-----	EKSRLASEEQ	ILRAATSIYG	APGQAEPPQA	FIDEVAKVYE	INHGRGPNQE	-----
-----	-----	-K-----	-----	-----	-----	-----
-----	-----	-----	-----	-----	-----	-G-----
-----	-----	-----	-----	-----	-----	-----
-----	-----	-----	-----	-----	-----	-----
-----	-D-----	-----	-----	-----	-----	-----
-----	-----	-K-----	-----	-----	-----	-----
-----	-----	-----	-----	-----	-----	-----
-P-----	-----	-----	-----	-R-----	-T-----	-V-----

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FIG. 31

	10	20	30	40	50	
GLS	QMKDLLLTAM	EMKHRNPRRA	PPKPKPRPNA	PTQRPGRLG	RWIRTVSDED	LE - 1012
DS326	-----	-----	-----K-----	-----	-----	---
E/De1	-----	-----	-----K-----	-----	-----	---
D78	-----	-----	-----L-----	-----	-----	---
Cu-1	-----	-----	-----L-----	-----	-----	---
PBG98	-----	-----	-----L-----	-----	-----	---
52/70	-----	-----	-----K-----	-----	-----	---
STC	-----	-----L-----	-----K-----	-----	-----	---
002-73	-----	-----	-----K-----	-----S-----	-----	---
OH	-----	-----	-----K-----	-----S-----	-----	---

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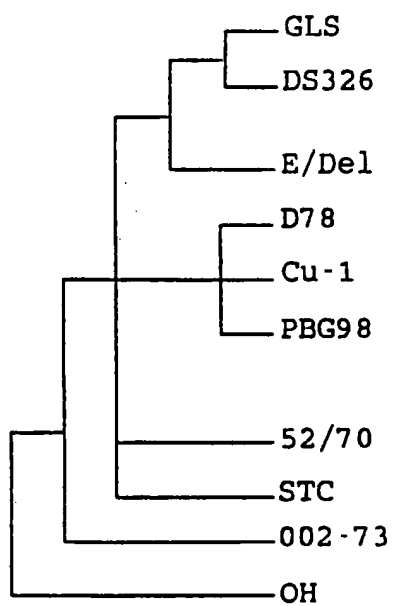
FIG. 4

FIG. 5A

 * TRANSLATION OF A NUCLEIC ACID SEQUENCE *

Done on large genome segment A of GLS-IBDV.

DE From cDNA clones pGLS-1 to pGLS-4.

Total number of bases is: 3230.

Analysis done on bases 114 to 3152.

Done on (absolute) phase(s): 3.

Using the Universal genetic code.

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CC CCG

10	20	30	40	50	
GGG GAG TCA CCC GGG GAC AGG CCG TCA AGG CCT TGT TCC AGG ATG GAA CTC CCC					
60	70	80	90	100	110
CTT CTA CAA TGC TAT CAT TGA TGG TTA GTA GAG ATC GGA CAA ACG ATC GCA GCG					
120	130	140	150	160	
ATG ACA AAC CTG CAA GAT CAA ACC CAA CAG ATT GTT CCG TTC ATA CGG AGC CTT					
MET Thr Asn Leu Gln Asp Gln Thr Gln Gln Ile Val Pro Phe Ile Arg Ser Leu					

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FIG. 5C

500	510	520	530	540
GTG ACC TTC CAA GGA AGC CTG AGT GAA CTG ACA GAT GTT AGC TAC AAT GGG TTG				
Val Thr Phe Gln Gly Ser Leu Ser Glu Leu Thr Asp Val Ser Tyr Asn Gly Leu				
550	560	570	580	590
ATG TCT GCA ACA GCC AAC ATC AAC GAC AAC ATT GGG AAC GTC CTA GTA GGG GAA				
MET Ser Ala Thr Ala Asn Ile Asn Asp Lys Ile Gly Asn Val Leu Val Gly Glu				
600	610	620	630	640
GGG GTT ACT GTC CTC AGC TTA CCC ACA TCA TAT GAT CTT GGG TAT GTG AGG CTT				
Gly Val Thr Val Leu Ser Leu Pro Thr Ser Tyr Asp Leu Gly Tyr Val Arg Leu				
660	670	680	690	700
GGT GAC CCC ATA CCC GCT ATA GGG CTT GAC CCA AAA ATG GTA GCA ACA TGT GAC				
Gly Asp Pro Ile Pro Ala Ile Gly Leu Asp Pro Lys MET Val Ala Thr Cys Asp				
710	720	730	740	750
AGC AGT GAC AGG CCC AGA GTC TAC ACC ATA ACT GCA GCT GAT GAT TAC CAA TTC				
Ser Ser Asp Arg Pro Arg Val Tyr Thr Ile Thr Ala Ala Asp Asp Tyr Gln Phe				
770	780	790	800	810
TCA TCA CAG TAC CAA ACA GGT GGG GTA ACA ATC ACC CTG TTC TCA GCC AAC ATT				
Ser Ser Gln Tyr Gln Thr Gly Gly val Thr Ile Thr Leu Phe Ser Ala Asn Ile				

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FIG. 5D

820	GAT GCC ATC ACA AGC CTC AGC GTT GGG GGA GAG CTC GTG TTT AAA ACA AGC GTC	830	Asp Ala Ile Thr Ser Leu Ser Val Gly Gly Glu Leu Val Phe Lys Thr Ser Val	840		850		860		
870	CAC AGC CTT GTA CTG GGC GCC ACC ATC TAC CTT ATA GGC TTT GAT GGG TCT GCG	880	His Ser Leu Val Leu Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Ser Ala	890		900		910		920
930	GTA ATC ACT AGA GCT GTG GCC GCA AAC AAT GGG CTG ACG ACC GGC ACC GAC AAT	940	Val Ile Thr Arg Ala Val Ala Val Ala Asn Asn Gly Leu Thr Thr Gly Thr Asp Asn	950		960		970		
980	CTT ATG CCA TTC AAT CTT GTG ATT CCA ACC AAC GAG ATA ACC CAG CCA ATC ACA	990	Leu MET Pro Phe Asn Leu Val Ile Pro Thr Asn Glu Ile Thr Gln Pro Ile Thr	1000		1010		1020		1030
1040	TCC ATC AAA CTG GAG ATA GTG ACC TCC AAA AGT GGT GGT CAG GAA GGG GAC CAG	1050	Ser Ile Lys Leu Glu Ile Val Thr Ser Lys Ser Gly Gly Gln Glu Gly Asp Gln	1060		1070		1080		
1090	ATG TCA TGG TCG GCA AGT GGG AGC CTA GCA GTG ACG ATT CAT GGT GGC AAC TAT	1100	MET Ser Trp Ser Ala Ser Gly Ser Leu Ala Val Thr Ile His Gly Gly Asn Tyr	1110		1120		1130		

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FIG. 5E

1140	1150	1160	1170	1180	1190
CCA GGG GCC CTC	CGT CCC GTC	ACA CTA GTA GCC	TAC GAA AGA	GTG GCA ACA	GGA
Pro Gly Ala Leu	Arg Pro Val	Thr Leu Val Ala	Tyr Glu Arg	Val Ala Thr	Gly
1200	1210	1220	1230	1240	
TCT GTC GTT ACG	GTC GCT GGG	GTG AGC AAC	TTC GAG CTG	ATC CCA AAT	CCT GAA
Ser Val Val Thr	Val Ala Gly	Val Ser Asn	Phe Glu Leu	Ile Pro Asn	Pro Glu
1250	1260	1270	1280	1290	1300
CTA GCA AAG AAC	CTG GTT ACA	GAA TAC GGC	CGA TTT GAC	CCA GGA	GGC ATG AAC
Leu Ala Lys Asn	Leu Val Thr	Glu Thr Gly	Arg Phe Asp	Pro Gly Ala	MET Asn
1310	1320	1330	1340	1350	
TAC ACA AAA TTG	ATA CTG AGT	GAG AGG GAC	CGC CTT GGC	ATC AAG	ACA GTC TGG
Tyr Thr Lys Leu	Ile Leu Ser	Glu Arg Asp	Arg Leu gly	Ile Lys Thr	Val Trp
1360	1370	1380	1390	1400	
CCG ACA AGG GAG	TAC ACC GAC	TTT CGT GAG	TAC TTC ATG	GAG GTG	GCC GAC CTC
Pro Thr Arg Glu	Tyr Thr Asp	Phe Arg Glu	Tyr Phe MET	Glu Val Ala	Asp Leu
1410	1420	1430	1440	1450	1460
AGC TCT CCC CTG	AAG ATT GCA	GGA GCA TTT	GGC TTC AAA	GAC ATA	ATC CGG GCC
Ser Ser Pro Leu	Lys Ile Ala	Gly Ala Phe	Gly Phe Lys	Asp Ile	Arg Ala

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FIG. 5F

1470	1480	1490	1500	1510	
ATA AGG AGG ATA GCT GTG CCG GTG GTC TCC ACA TTG TTC CCA CCT GCC GCT CCC	Ile Arg Arg Ile Ala Val Pro Val Val Ser Thr Leu Phe Pro Pro Ala Ala Pro				
1520	1530	1540	1550	1560	1570
CTG GCC CAT GCA ATT GGG GAA GGT GTA GAC TAC CTG CTG GGT GAT GAG GCA CAG	Leu Ala His Ala Ile Gly Glu Gly Val Asp Tyr Leu Leu Gly Asp Glu Ala Gln				
1580	1590	1600	1610	1620	
GCT GCT TCA GGA ACT GCT CGA GCC GCG TCA GGA AAA GCA AGG GCT GCC TCA GGC	Ala Ala Ser Gly Thr Ala Arg Ala Ala Ser Gly Lys Ala Arg Ala Ser Gly				
1630	1640	1650	1660	1670	
CGC ATA AGG CAG CTG ACT CTC GCG GCC GCG GAC AAG GGG TAC GAG GTA GTC GCG AAT	Arg Ile Arg Gln Leu Thr Leu Ala Ala Asp Lys Gly Tyr Glu Val Val Ala Asn				
1680	1690	1700	1710	1720	1730
CTA TTC CAG GTG CCC CAG AAT CCC GTA GTC GAC GGG ATT CTT GCT TCA CCC GGG	Leu Phe Gln Val Pro Gln Asn Pro Val Val Asp Gly Ile Leu Ala Ser Pro Gly				
1740	1750	1760	1770	1780	
ATA CTC CGC GGT GCA CAC AAC CTC GAC TGC GTG TTA AGA GAG GGC GCC ACG CTA	Ile Leu Arg Gly Ala His Asn Leu Asp Cys Val Leu Arg Glu Gly Ala Thr Leu				

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FIG. 5C

1790	1800	1810	1820	1830	1840
TTC CCT GTG GTC ATC ACG ACA GTG GAA GAC GCC ATG ACA CCC AAA GCA CTA AAC					
Phe Pro Val Val Ile Thr Thr Val Glu Asp Ala MET Thr Pro Lys Ala Leu Asn					
1850	1860	1870	1880	1890	
AGC AAA ATG TTT GCT GTC ATT GAA GGC GTG CGA GAG GAC CTC CAA CCT CCA TCT					
Ser Lys MET Phe Ala Val Ile Glu Gly Val Arg Glu Asp Leu Gln Pro Pro Ser					
1900	1910	1920	1930	1940	
CAA AGA GGA TCC TTC ATA CGA ACT CTC TCC GGA CAC AGA GTC TAT GGA TAT GCT					
Gln Arg Gly Ser Phe Ile Arg Thr Leu Ser Gly His Arg Val Tyr Gly Tyr Ala					
1950	1960	1970	1980	1990	2000
CCA GAT GGG GTA CTT CCA CTG GAG ACT GGG AGA GAC TAC ACC GTT GTC CCA ATA					
Pro Asp Gly Val Leu Pro Leu Glu Thr Gly Arg Asp Tyr Thr Val Val Pro Ile					
2010	2020	2030	2040	2050	
GAT GAT GTC TGG GAC GAC AGC ATT ATG CTG TCC AAA GAC CCC ATA CCT CCT ATT					
Asp Asp Val Trp Asp Asp Ser Ile MET Leu Ser Lys Asp Pro Ile Pro Pro Ile					
2060	2070	2080	2090	2100	2110
GTG GGA AAC AGT GGA AAC CTA GCC ATA GCT TAC ATG GAT GTG TTT CGA CCC AAA					
Val Gly Asn Ser Gly Asn Leu Ala Ile Ala Tyr MET Asp Val Phe Arg Pro Lys					

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FIG. 5H

2120	2130	2140	2150	2160
GTC CCC ATC CAT GTG GCC ATG ACG GGA GCC CTC AAC GCT TGT GGC GAG ATT GAG				
Val Pro Ile His Val Ala MET Thr Gly Ala Leu Asn Ala Cys Gly Glu Ile Glu				
2170	2180	2190	2200	2210
AAA ATA AGC TTT AGA AGC ACC AAG AAG CTC GGC ACC GCA CAC CGG CTT GGC CTC AAG				
Lys Ile Ser Phe Arg Ser Thr Lys Leu Ala Thr Ala His Arg Leu Gly Leu Lys				
2220	2230	2240	2250	2260
TTG GCT GGT CCC GGA GCA TTT GAT GTA AAC ACC GGG CCC AAC TGG GCA ACG TTC				
Leu Ala Gly Pro Gly Ala Phe Asp Val Asn Thr Gly Pro Asn Trp Ala Thr Phe				
2280	2290	2300	2310	2320
ATC AAA CGT TTC CCT CAC AAT CCA CGC GAC TGG GAC AGG CTC CCC TAC CTC AAC				
Ile Lys Arg Phe Pro His Asn Pro Arg Asp Trp Asp Arg Leu Pro Tyr Leu Asn				
2330	2340	2350	2360	2370
CTT CCA TAC CTT CCA CCC AAT GCA GGA CGC CAG TAC CAC CTC GCC ATG GCC GCA				
Leu Pro Tyr Leu Pro Pro Asn Ala Gly Arg Gln Tyr His Leu Ala MET Ala Ala				
2390	2400	2410	2420	2430
TCA GAG TTC AAG GAG ACC CCT GAA CTC GAG AGC GCC GTC AGG GCC ATG GAA GCA				
Ser Glu Phe Lys Glu Thr Pro Glu Leu Glu Ser Ala Val Arg Ala MET Glu Ala				

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FIG. 51

2440	245	2460	2470	2480
GCA GCC AGT GTA GAC CCA CTG TTC CAA TCT GCA CTC AGT GTG TTC ATG TGG CTG				
Ala Ala Ser Val Asp Pro Leu Phe Gln Ser Ala Leu Ser Val Phe MET Trp Leu				
2490	2500	2510	2520	2530
GAA GAG AAT GGG ATT GTG ACT GAC ATG GCC AAC TTC GCA CTC AGC GAC CCG AAC				
Glu Glu Asn Gly Ile Val Thr Asp MET Ala Asn Phe Ala Leu Ser Asp Pro Asn				
2540	2550	2560	2570	2580
GCC CAT CGG ATG CGA AAC TTT CTT GCA AAC GCA CCA CAA GCA GGT AGC AAG TCT				
Ala His Arg MET Arg Asn Phe Leu Ala Asn Ala Pro Gln Ala Gly Ser Lys Ser				
2590	2600	2610	2620	2630
CAA AGG GCC AAA TAC GGG ACA GCA GGC TAC GGA GTG GAG GCC CGG GGC CCC ACA				
Gln Arg Ala Lys Tyr Gly Thr Ala Gly Tyr Gly Val Glu Ala Arg Gly Pro Thr				
2640	2650	2660	2670	2680
CCA GAA GAA GCA CAG AGG GAA AAA GAC ACA CGG ATC TCA AAG AAG ATG GAG ACC				
Pro Glu Glu Ala Gln Arg Glu Lys Asp Thr Arg Ile Ser Lys Lys MET Glu Thr				
2690	2700	2710	2720	2730
ATG GGC ATC TAC TTT GCA ACA CCA GAA TGG GTA GCA CTC AAT GGG CAC CGA GGG				
MET Gly Ile Tyr Phe Ala Thr Pro Glu Trp Val Ala Leu Asn Gly His Arg Gly				

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FIG. 5K

3090	3100	3110	3120	3130
CCA ACG CAG AGA CCC CCT GGT CGG CTG GGC CGC TGG ATC AGG ACT GTC TCT GAT				
Pro Thr Gln Arg Pro Pro Gly Arg Leu Gly Arg Trp Ile Arg Thr Val Ser Asp				
3140	3150	3160	3170	3180
GAG GAC CTT GAG TGA GGC TCC TGG GAG TCT CCC GAC ACC ACC CGC GCA GGC GTG				
Glu Asp Leu Glu ---				
3200	3210	3220	3230	
GAC ACC AAT TCG GCC TTA CAA CAT CCC AAA TTG GAT CCG				
---2 Aug - 1990 -----PC/Gene---				

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FIG. 6B

170	180	190	200	210	
GAG AAG CAC ACT CTC AGG TCA GAG ACC TCG ACC TAC AAT TTG ACT GTG GGG GAC					
Glu Lys His Thr Leu Arg Ser Glu Thr Ser Thr Tyr Asn Leu Thr Val Gly Asp					
220	230	240	250	260	270
ACA GGG TCA GGG CTA ATT GTC TTT TTC CCT GGA TTC CCT GGC TCA ATT GTG GGT					
Thr Gly Ser Gly Leu Ile Val Phe Phe Pro Gly Phe Pro Gly Ser Ile Val Gly					
280	290	300	310	320	
GCT CAC TAC ACA CTG CAG AGC AGT GGG AAC TAC AAG TTC GAT CAG ATG CTC CTG					
Ala His Tyr Thr Leu Gln Ser Ser Gly Asn Tyr Lys Phe Asp Gln MET Leu Leu					
330	340	350	360	370	
ACT GCC CAG AAC CTA CCG GCC AGC TAC AAC TAC TGC AGG CTA GTG AGT CGG AGT					
Thr Ala Gln Asn Leu Pro Ala Ser Tyr Asn Tyr Cys Arg Leu Val Ser Arg Ser					
380	390	400	410	420	430
CTC ACA GTA AGG TCA AGC ACA CTC CCT GGT GGC GTT TAT GCA CTA AAC GGC ACC					
Leu Thr Val Arg Ser Thr Leu Pro Gly Gly Val Tyr Ala Leu Asn Gly Thr					

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FIG. 6C

440	450	460	470	480
ATA AAC GCC GTG ACC TTC CAA GGA AGC CTG AGT GAA CTG ACA GAT GTT AGC TAC				
Ile Asn Ala Val Thr Phe Gln Gly Ser Leu Ser Glu Leu Thr Asp Val Ser Tyr				
490	500	510	520	530
AAC GGG TTG ATG TCT GCA ACA GCC AAC ATC AAC GAC AAA ATT GGG AAC GTC CTA				
Asn Gly Leu MET Ser Ala Thr Ala Asn Ile Asn Asp Lys Ile Gly Asn Val Leu				
540	550	560	570	580
GTA GGG GAA GGG GTA ACC GTC CTC AGC TTA CCC ACA TCA TAT GAT CTT CCG TAT				
Val Gly Glu Gly Val Thr Val Thr Leu Ser Leu Pro Thr Ser Tyr Asp Leu Gly Tyr				
590	600	610	620	630
GTG AGG CTT GGT GAC CCC ATA CCC GCT ATA GGG CTT GAC CCA AAA ATG GTA GCA				
Val Arg Leu Gly Asp Pro Ile Pro Ala Ile Gly Leu Asp Pro Lys MET Val Ala				
640	650	660	670	680
ACA TGT GAC AGC AGT GAC AGG CCC AGA GTC TAC ACC ATA ACT GCA GCC GAT AAT				
Thr Cys Asp Ser Ser Asp Arg Pro Arg Pro Arg Val Tyr Thr Ile Thr Ala Ala Asp Asn				
690	700			

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FIG. 6D

710	720	730	740	750	
TAC CAA TTC TCA TCA CAG TAC CAA ACA GGT GGG GTA ACA ATC ACA CTG TTC TCA					
Tyr Gln Phe Ser Ser Gln Tyr Gln Thr Gly Gly Val Thr Ile Thr Leu Phe Ser					
760	770	780	790	800	810
GCC AAC ATT GAT GCC ATC ACA AGT CTC AGC GTT GGG GGA GAG CTC GTG TTC AAA					
Ala Asn Ile Asp Ala Ile Thr Ser Leu Ser Val Gly Gly Glu Leu Val Phe Lys					
820	830	840	850	860	
ACA AGC GTC CAA AGC CTT GTA CTG GGC GCC GCC ACC ATC TAC CTT ATA GGC TTT GAT					
Thr Ser Val Gln Ser Leu Val Leu Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp					
870	880	890	900	910	
GGG ACT GCG GTA ATC ACC AGA GCT GTG GCC GCA AAC AAT GGG CTG ACG GCC GGC					
Gly Thr Ala Val Ile Thr Arg Ala Val Ala Ala Asn Asn Gly Leu Thr Ala Gly					
920	930	940	950	960	970
ATC GAC AAT CTT ATG CCA TTC AAT CTT GTG ATT CCA ACC AAT AAT GAG ATA ACC CAG					
Ile Asp Asn Leu MET Pro Phe Asn Leu Val Ile Pro Thr Asn Glu Ile Thr Gln					

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FIG. 6E

980	CCA ATC ACA	990	TCC ATC ATA	1000	CTG GAG ATA GTG	1010	ACC TCC AAA	1020	AGT GAT GGT		CAG GCA
	Pro Ile Thr Ser Ile Ile		Ile Ile		Val Val		Thr Ser Lys Ser		Asp Gly Gln Ala		
1030	GGG GAA CAG	1040	ATG TCA	1050	TGG TCG GCA	1060	AGT GGG AGC	1070	CTA GCA GTG	1080	ACG ATC CAT GGT
	Gly Glu Gln MET Ser Trp Ser		Trp Ser		Ala Ser		Gly Ser Leu Ala Val		Thr Ile His Gly		
1090	GGC AAC TAT	1100	CCA GGA	1110	GCC CTC CGT	1120	CCC GTC ACA	1130	CTA GTG GCC		TAC GAA AGA GTG
	Gly Asn Tyr Pro Gly Ala Leu Arg		Pro Val		Thr Leu Val Ala Tyr Glu Arg Val						
1140	GCA ACA GGA	1150	TCT GTC	1160	GTT ACG	1170	GTC GCT GGG	1180	GTG AGC AAC		TTC GAG CTG ATC CCA
	Ala Thr Gly Ser Val Val Thr Val Ala Gly Val Ser Asn Phe Glu Leu Ile Pro										
1190	AAT CCT GAA	1200	CTA GCA	1210	AAG AAC	1220	CTG GTT	1230	ACA GAA TAC	1240	GAC CCA GGA
	Asn Pro Glu Leu Ala Lys Asn Leu Val Thr Glu Tyr Gly Arg Phe Asp Pro Gly										

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FIG. 6F

1250	1260	1270	1280	1290
GCC ATG AAC TAC ACG AAA TTG ATA CTG AGT GAG AGG GAC CAC CTT GGC ATC AAG				
Ala MET Asn Tyr Thr Lys Lys Leu Ile Leu Ser Glu Arg Asp His Leu Gly Ile Lys				
1300	1310	1320	1330	1340
ACC GTC TGG CCA ACA AGG GAG TAC ACT GAC TTT CGT GAG TAC TTC ATG GAG GTG				
Thr Val Trp Pro Thr Arg Glu Tyr Thr Asp Phe Arg Glu Tyr Phe MET Glu Val				
1360	1370	1380	1390	1400
GCC GAC CTC AAC TCT CCC CTG AAG ATT GCA GGA GCA TTT GGC TTC AAA GAG ATA				
Als Asp Leu Asn Ser Pro Leu Lys Ile Ala Gly Ala Phe Gly Phe Lys Asp Ile				
1410	1420	1430	1440	1450
ATC CGG GCC ATA AGG AGG ATA GCT GTA CCG GTG GTC TCT ACA TTG TTC CCA CCT				
Iel Arg Ala Ile Arg Arg Ile Ala Val Pro Val Val Ser Thr Leu Phe Pro Pro				
1460	1470	1480	1490	1500
GCC GCT CCT CTA GCC CAT GCA ATT GGG GAA GGT GTA GAC TAC CTA CTG GGC GAT				
Ala Ala Pro Leu Ala His Ala Ile Gly Glu Gly Val Asp Tyr Leu Leu Gly Asp				

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FIG. 6C

1520	1530	1540	1550	1560
GAG GCA CAG GCT GCT TCA GGA ACC GCT CGA GCC GCG TCA GGA AAA GCA AGG GCT	Glu Ala Gln Ala Ala Ser Gly Thr Ala Arg Ala Ala Ser Gly Lys Ala Arg Ala			
1570	1580	1590	1600	1610
GCC TCA GGC CGC ATA AGG CAG CTG ACT CTC GCC GCC GAC AAG GGG TAC GAG GTA	Ala Ser Gly Arg Ile Arg Gln Leu Thr Leu Ala Ala Asp Lys Gly Tyr Glu Val			
1630	1640	1650	1660	1670
GTC GCG AAT CTA TTC CAG GTG CCC CAG AAT CCC GTA GTC GAC GGG ATT CTT GCT	Val Ala Asn Leu Phe Gln Val Pro Gln Asn Pro Val Val Asp Gly Ile Leu Ala			
1680	1690	1700	1710	1720
TCA CCC GGG ATA CTT CGC GGT GCA CAC AAC CTC GAC TGC GTG CTA AGA GAG GGT	Ser Pro Gly Ile Leu Arg Gly Ala His Asn Leu Asp Cys Val Leu Arg Glu Gly			
1730	1740	1750	1760	1770
GCC ACG CTA TTC CCT GTG GTC ATT ACG ACA GTG GAA GAC GCC ATG ACA CCC AAA	Ala Thr Leu Phe Pro Val Val Ile Thr Thr Val Glu Asp Ala MET Thr Pro Lys			

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FIG. 6H

1790	1800	1810	1820	1830	
GCA CTG AAC AGC AAA ATG TTT GCT GTC ATT GAA GGC GTG CGA GAA GAC CTC CAA					
Ala Leu Asn Ser Lys MET Phe Ala Val Ile Glu Gly Val Arg Glu Asp Leu Gln					
1840	1850	1860	1870	1880	1890
CCT CCA TCT CAA AGA GGA TCC TTC ATA CGA ACT CTC TCC GGA CAC AGA GTC TAT					
Pro Pro Ser Gln Arg Gly Ser Phe Ile Arg Thr Leu Ser Gly His Arg Val Tyr					
1900	1910	1920	1930	1940	
GGA TAT GCT CCA GAT GGG GTA CTT CCA CTG GAG ACT GGG AGA GAC TAC ACC GTT					
Gly Tyr Ala Pro Asp Gly Val Leu Pro Leu Glu Thr Gly Arg Asp Tyr Thr Val					
1950	1960	1970	1980	1990	
GTC CCA ATA GAT GAT GTC TGG GAC GAC AGC ATT ATG CTG TCC AAG GAC CCC ATA					
Val Pro Ile Asp Asp Val Trp Asp Asp Ser Ile MET Leu Ser Lys Asp Pro Ile					
2000	2010	2020	2030	2040	2050
CCT CCT ATT GTG GGA AAC AGT GGA AAC CTA GCC ATA GCT TAC ATG GAT GTG TTT					
Pro Pro Ile Val Gly Asn Ser Gly Asn Leu Ala Ile Ala Tyr MET Asp Val Phe					

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FIG. 6I

2060	2070	2080	2090	2100
CGA CCC AAA GTC CCC ATC CAT GTG GCC ATG ACG GGA GCC CTC AAC GCT TGT GGC				
Arg Pro Lys Val Pro Ile His Val Ala MET Thr Gly Ala Leu Asn Ala Cys Gly				
2110	2120	2130	2140	2150
GAG ATT GAG AAA ATA AGC TTC AGA AGC ACC AAG CTC GCC ACC GCA CAC CGG CTT				
Glu Ile Glu Lys Ile Ser Phe Arg Ser Thr Lys Leu Ala Thr Ala His Arg Leu				
2170	2180	2190	2200	2210
GGC CTC AAG TTG GCT GGT CCC GGA GCA TTC GAT GTA AAC ACC GGG CCC AAC TGG				
Gly Leu Lys Lys Leu Ala Gly Pro Gly Ala Phe Asp Val Asn Thr Gly Pro Asn Trp				
2220	2230	2240	2250	2260
GCA ACG TTC ATC AAA CGT TTC CCT CAC AAT CCA CGC GAC TGG GAC AGG CTC CCC				
Ala Thr Phe Ile Lys Arg Phe Pro His Asn Pro Arg Asp Trp Asp Arg Leu Pro				
2270	2280	2290	2300	2310
TAC CTC AAC CTT CCA TAC TAC CTT CCA CCC AAT GCA GGA CGC CAG TAC CAC CTT GCC				
Tyr Leu Asn Leu Pro Tyr Leu Pro Pro Asn Ala Gly Arg Gln Tyr His Leu Ala				

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FIG. 6J

2330	2340	2350	2360	2370	
ATG GCT GCA TCA GAG TTT AAA GAG ACC CCT GAA CTC GAG AGC GCC GTT AGA GCC					
MET Ala Ala Ser Glu Phe Lys Glu Thr Pro Glu Leu Ser Ala Val Arg Ala					
2380	2390	2400	2410	2420	2430
ATG GAA GCA GCA GCC AAT GTG GAC CCA CTG TTC CAA TCT GCA CTC AGT GTG TTC					
MET Glu Ala Ala Ala Asn Val Asp Pro Leu Phe Gln Ser Ala Leu Ser Val Phe					
2440	2450	2460	2470	2480	
ATG TGG CTG GAA GAG AAT GGG ATT GTG GCT GAC ATG GCC AAT TTC GCA CTC AGC					
MET Trp Leu Glu Glu Asn Gly Ile Val Ala Asp MET Ala Asn Phe Ala Leu Ser					
2490	2500	2510	2520	2530	
GAC CCG AAC GCC CAT CGG ATG CGA AAT TTT CTT GCA AAC GCA CCA CAA GCA GGC					
Asp Pro Asn Ala Ala His Arg MET Arg Asn Phe Leu Ala Asn Ala Pro Gln Ala Gly					
2540	2550	2560	2570	2580	2590
AGC AAG TCG CAA AGG GCC AAG TAC GGG ACA GCA GGC TAC GGA GTG GAG GCC CGG					
Ser Lys Ser Gln Arg Ala Lys Tyr Gly Thr Ala Gly Tyr Gly Val Glu Ala Arg					

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FIG. 6K

2600	2610	2620	2630	2640
GGC CCC ACA CCA GAG GAA GCA CAG AGG GAA AAA GAC ACA CGG ATC TCA AAG AAG				
Gly Pro Thr Pro Glu Glu Ala Gln Arg Glu Lys Asp Thr Arg Ile Ser Lys Lys				
2650	2660	2670	2680	2690
ATG GAG ACC ATG GGC ATC TAC TTT GCA ACA CCA GAA TGG GTA GCA CTC AAT GGG				
MET Glu Thr MET Gly Ile Tyr Phe Ala Thr Pro Glu Trp Val Ala Leu Asn Gly				
2710	2720	2730	2740	2750
CAC CGA GGG CCA AGC CCC GGC CAG CTA AAG TAC TGG CAG AAC ACA CGA GAA ATA				
His Arg Gly Pro Ser Pro Gly Gln Leu Lys Tyr Trp Gln Asn Thr Arg Glu Ile				
2760	2770	2780	2790	2800
CCG GAC CCA AAC GAG GAC TAT CTA GAC TAC GTG CAT GCA GAG AAG AGC CGG TTG				
Pro Asp Pro Asn Glu Asp Tyr Leu Asp Tyr Val His Ala Glu Lys Ser Arg Leu				
2810	2820	2830	2840	2850
GCA TCA GAA GAA CAA ATC CTA AAG GCA GCT ACG TCG ATC TAC GGG GCT CCA GGA				
Ala Ser Glu Glu Gln Ile Leu Lys Ala Ala Thr Ser Ile Tyr Gly Ala Pro Gly				
				2860

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FIG. 6L

2870	2880	2890	2900	2910	
CAG GCA GAG	CCA CCC CAA	GCT TTC ATA	GAC GAA GTT	GCC AAA GTC	TAT GAA ATC
Gln Ala Glu	Pro Pro Gln	Ala Phe Ile	Asp Glu Val	Ala Lys Val	Tyr Glu Ile
2920	2930	2940	2950	2960	2970
AAC CAT GGA	CGT GGC CCT	AAC CAA GAA	CAG ATG AAA	GAT CTG CTC	TTG ACT GCA
Asn His Gly	Arg Gly Pro	Asn Gln Glu	Gln MET Lys	Asp Leu Leu	Leu Thr Ala
2980	2990	3000	3010	3020	
ATG GAG ATG	AAG CAT CGC	AAC CGG AGG	CGT CCA CCA	AAG CCC AAG	CCA AAA
MET Glu MET	Lys His Arg	Asn Pro Arg	Ala Pro Pro	Lys Pro Lys	Pro Lys
3030	3040	3050	3060	3070	
CCC AAT GCT	CCA ACA CAG	AGA CCT GGT	CGG CTG GGC	CGC TGG	ATC AGG ACC
Pro Asn Ala	Pro Thr Gln	Arg Pro Pro	Gly Arg Leu	Gly Arg Trp	Ile Arg Thr
3080	3090	3100	3110	3120	3130
GTC TCT GAT	GAG GAC CTT	GAG TGA GGC	CCC TGG	GGG TCT	CCC ACC ACC
Val Ser Asp	Glu Asp Leu	Glu Glu	---	---	---

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FIG. 6M

```
3140      |      3150      |      3160      |      3170      |      3180      |
GCA GGC GTG GAC ACC AAT TCG GCC TTA CAA CAT CCC AAA TTG GAT CCG
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===24-AUG-1992=====PC/GENE===
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FIG. 7A

AMINO ACID CHANGES IN VP2 VARIOUS IBDV STRAINS

VIRUSES	AMINO ACID RESIDUE NUMBER IN VP2															
	5	74	76	80	213	222	239	242	249	253	254	258	263	264	269	270
GLS	Gln	Leu	Ser	Tyr	Asp	Thr	Ser	Val	Lys	His	Ser	Gly	Leu	Ile	Ser	Ala
SD326	"	"	"	"	"	Ser	"	"	"	Gln	"	"	"	"	Thr	"
E/DEL	"	"	"	"	Asn	Thr	"	"	"	"	"	"	"	"	"	"
D78	"	"	Gly	"	Asp	Pro	"	"	Gln	His	Gly	"	"	"	"	Thr
Cu-1	"	"	Ser	"	"	"	"	"	"	"	"	"	"	"	"	"
PBC98	"	"	"	"	"	"	"	"	Arg	"	"	"	"	"	"	"
52/70	"	"	"	"	"	"	"	Ile	Gln	Gln	"	"	"	"	"	Ala
STC	"	"	"	Leu	"	"	"	Val	"	"	"	"	Phe	"	"	Thr
002-73	Ser	Met	"	Tyr	"	"	Asn	"	"	"	"	Asn	Leu	Val	"	"
																Gly
																Thr
																Ile
																Asn

FIG. 7B

VIRUSES	AMINO ACID RESIDUE NUMBER IN VP2															
	280	284	286	279	299	305	311	312	318	320	321	323	326	328	330	332
GLS	Asn	Thr	Thr	Pro	Asn	Ile	Glu	Ile	Gly	Gln	Glu	Asp	Ser	Ser	Ser	Ser
SD326	"	Ala	"	"	"	"	Lys	"	"	Leu	"	"	"	"	"	Asn
E/DEL	"	"	Ile	"	"	"	Glu	"	Asp	Gln	Ala	Glu	"	"	"	"
D78	"	Thr	Thr	"	"	"	"	"	Gly	"	"	Asp	"	"	Arg	"
Cu-1	"	"	"	Ser	"	"	"	"	"	"	"	"	"	"	Lys	"
PBC98	Thr	"	"	Pro	"	"	"	"	"	"	"	"	Leu	"	Arg	"
52/70	Asn	Ala	"	"	"	"	"	"	"	"	"	"	Ser	"	Ser	"
STC	"	"	"	"	"	"	"	Lys	"	"	"	"	"	"	"	"
002-73	"	"	"	"	Ser	Val	"	Glu	"	"	"	"	"	Leu	"	Asn

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/03772

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/159.1, 185.1, 186.1, 204.1; 435/320.1, 252.3; 530/350, 388.3, 397, 402, 403; 935/10, 12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, CA, BIOSIS, CABA

SEARCH TERMS: IBDV, INFECTIOUS BURSAL DISEASE VIRUS, VP2, VP4; VP3, ANTIBOD?, ASP, ASPARTIC ACID, VACCIN?, DNA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	JOURNAL OF GENERAL VIROLOGY, Volume 74, issued 1993, V.N. Vakharia et al., "Infectious Bursal Disease Virus Structural Proteins Expressed in a Baculovirus Recombinant Confer Protection in Chickens", pages 1201-1206, see entire document.	1-8, 10-13, 18-20 ----- 9
X -- Y	ARCHIVES OF VIROLOGY, Volume 120, issued 1991, C.D. Bayliss et al., "A Recombinant Fowlpox Virus that Expresses the VP2 Antigen of Infectious Disease Virus Induces Protection Against Mortality Caused by the Virus", pages 193-205, see entire document.	1-4 ----- 8, 10, 18-20

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/03772

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF GENERAL VIROLOGY, Volume 70, issued 1989, K.J. Fahey et al., " A Conformational Immunogen on VP-2 of Infectious Bursal Disease Virus that Induces Virus-Neutralizing Antibodies That Passively Protect Chickens", pages 1473-1481, see entire document.	14-17

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/03772

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A61K 35/76, 39/12, 39/395; C07K 14/005, 16/08; C12N 1/21, 5/10, 15/33

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/159.1, 185.1, 186.1, 204.1; 435/320.1, 252.3; 530/350, 388.3, 397, 402, 403; 935/10, 12

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